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NATIONAL NUCLEAR ENERGY SERIES
Manhattan Project Technical Section

Division VI - Volume 1

PHARMACOLOGY AND TOXICOLOGY OF URANIUM COMPOUNDS

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PHARMACOLOGY AND TOXICOLOGY OF URANIUM COMPOUNDS

With a Section on the Pharmacology and Toxicology of Fluorine and Hydrogen Fluoride

Edited by

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OF URANIUM COMPOUNDS

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FOREWORD

The United States program of development of atomic energy has been described by Major General L. R. Groves, who, as Commanding General of the War Department's Manhattan Project, directed the program from mid-1942 until December 31, 1946, as "a generation of scientific development compressed into three years." The tremendous scope of the Manhattan Project Technical Section of the National Nuclear Energy Series, which has been in preparation since 1944, is a tribute to the unprecedented accomplishments of science, industry, government, labor, and the Army and Navy working together as a team. These volumes can be a firm foundation for the United States atomic energy program which, in the words of the Atomic Energy Act of 1946, is " . . . directed toward improving the public welfare, increasing the standard of living, strengthening free competition in private enterprise, and promoting world peace."

David E. Lilienthal, Chairman
U. S. Atomic Energy Commission

ACKNOWLEDGMENT

The Manhattan Project Technical Section of the National Nuclear Energy Series embodies results of work done in the nation's wartime atomic energy program by numerous contractors, including Columbia University. The arrangements for publication of the series volumes were effected by Columbia University, under a contract with the United States Atomic Energy Commission. The Commission, for itself and for the other contractors who contributed to this series, wishes to record here its appreciation of this service of Columbia University in support of the national nuclear energy program.

PREFACE

This volume is one of a series which has been prepared as a record of the research work done under the Manhattan Project and the Atomic Energy Commission. The name Manhattan Project was assigned by the Corps of Engineers, War Department, to the far-flung scientific and engineering activities which had as their objective the utilization of atomic energy for military purposes. In the attainment of this objective, there were many developments in scientific and technical fields which are of general interest. The National Nuclear Energy Series (Manhattan Project Technical Section) is a record of these scientific and technical contributions, as well as of the developments in these fields which are being sponsored by the Atomic Energy Commission.

The declassified portion of the National Nuclear Energy Series, when completed, is expected to consist of some 60 volumes. These will be grouped into eight divisions, as follows:

Division I — Electromagnetic Separation Project
Division II — Gaseous Diffusion Project
Division III — Special Separations Project
Division IV — Plutonium Project
Division V — Los Alamos Project
Division VI — University of Rochester Project
Division VII — Materials Procurement Project
Division VIII — Manhattan Project

Soon after the close of the war the Manhattan Project was able to give its attention to the preparation of a complete record of the research work accomplished under Project contracts. Writing programs were authorized at all laboratories, with the object of obtaining complete coverage of Project results. Each major installation was requested to designate one or more representatives to make up a committee, which was first called the Manhattan Project Editorial Advisory Board, and later, after the sponsorship of the Series was assumed by the Atomic Energy Commission, the Project Editorial Advisory Board. This group made plans to coordinate the writing programs at all the installations, and acted as an advisory group in all matters affecting the Project-wide writing program. Its last meeting was held on Feb. 9, 1948, when it recommended the publisher for the Series.

The names of the Board members and of the installations which they represented are given below.

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University of Rochester	D. R. Charles, M. J. Wantman

* Represented Madison Square Area of the Manhattan District.

† The Y-12 plant at Oak Ridge was operated by Tennessee Eastman Corporation until May 4, 1947, at which time operations were taken over by Carbide & Carbon Chemicals Corporation.

‡ Clinton Laboratories was the former name of the Oak Ridge National Laboratory.

§ SAM (Substitute Alloy Materials) was the code name for the laboratories operated by Columbia University in New York under the direction of Dr. H. C. Urey, where much of the experimental work on isotope separation was done. On Feb. 1, 1945, the administration of these laboratories became the responsibility of Carbide & Carbon Chemicals Corporation. Research in progress there was transferred to the K-25 plant at Oak Ridge in June, 1946, and the New York laboratories were then closed.

Many difficulties were encountered in preparing a unified account of Atomic Energy Project work. For example, the Project Editorial Advisory Board was the first committee ever organized with representatives from every major installation of the Atomic Energy Project. Compartmentation for security was so rigorous during the war that it had been considered necessary to allow a certain amount of duplication of effort rather than to permit unrestricted circulation of research information between certain installations. As a result, the writing programs of different installations inevitably overlap markedly in many scientific fields. The Editorial Advisory Board has exerted itself to reduce duplication in so far as possible and to eliminate discrepancies in factual data included in the volumes of the NNES. In particular, unified Project-wide volumes have been prepared on Uranium Chemistry and on the Analysis of Project Materials. Nevertheless, the reader will find many instances of differences in results or conclusions on similar subject matter prepared by different authors. This has not seemed wholly undesirable for several reasons. First of all, such divergencies are not unnatural and stimulate investigation. Second, promptness of publication has seemed more important than the removal of all discrepancies. Finally, many Project scientists completed their contributions some time ago and have become engrossed in other activities so that their time has not been available for a detailed review of their work in relation to similar work done at other installations.

The completion of the various individual volumes of the Series has also been beset with difficulties. Many of the key authors and editors have had important responsibilities in planning the future of atomic energy research. Under these circumstances, the completion of this technical series has been delayed longer than its editors wished. The volumes are being released in their present form in the interest of presenting the material as promptly as possible to those who can make use of it.

The Editorial Advisory Board

UNIVERSITY OF ROCHESTER PROJECT FOREWORD

The University of Rochester Manhattan Project had its inception on April 5, 1943, with the appointment of Dr. Stafford L. Warren,* Professor of Radiology and Chairman of the Department of Radiology at the University of Rochester School of Medicine and Dentistry, as Consultant to the Manhattan Engineer District (later as Chief of the Medical Section). Under his guidance and direction the local project was established and its operational policies formulated. On November 2, 1943, Dr. Warren was commissioned colonel in the Army Medical Corps, and the subsequent responsibility for the Project was assumed by the present Director on November 13, 1943.

In many respects the atmosphere of the work was in marked contrast to the academic freedom of a university environment. The research was frequently of applied rather than of fundamental nature, though the latter was by no means lacking. In addition to physical and spiritual isolation from our accustomed confreres, we found ourselves surrounded by a multitude of security, Army, governmental, and war-manpower regulations, but the majority of the personnel made the necessary mental adjustments without undue hardship and with commendable reasonableness and good grace. Not infrequently we found these apparent handicaps working to our mutual advantage.

The organization of the Project was likewise unusual and, to a certain extent, experimental. To accomplish the task in the specified time and to utilize effectively experienced personnel made scarce by previous demands of the war, individuals were placed in positions where their capabilities could produce maximum benefit to the Project as a whole. To this end, ten autonomous but mutually interdependent divisions were established,† whose coordination was effected through the Director's Office so that priorities on material, manpower, and concentration of effort could be channeled in the proper direction with the shifting phases of the various problems. Experiments were discussed and organized on a cooperative basis through a system of

---

*Now Dean of the Medical School, University of California at Los Angeles.

†See Appendix for organization chart.

"planning sessions" in such a manner that the expert opinion of participating members of specialized divisions could make major contributions to the structure of many of the experiments. This procedure also enabled the divisions of Pathology, Hematology, and Statistics to coordinate their activities with other divisions so that they could analyze properly the mass of experimental data which must, of necessity, pass through their respective laboratories.

It would be misleading to aver that this system of research procedure presented here was without fault and not beset by difficulties. It is not an easy matter for an investigator to reconcile his ideas and personal ambitions with those of a group for a common objective, especially when frequently his entire training and previous progress have been based upon individual achievement. Particularly is this true when he has not had the privilege of participating in the selection of his associates. The system, however, worked surprisingly well under somewhat unfavorable circumstances and is worthy of further exploration under peacetime conditions.

It is impossible to pay proper tribute to the many individuals—scientific, technical, and nontechnical—who participated in this endeavor. Neither can one, by reading the following pages, appreciate fully the mental and physical labor that went into the enterprise. Approximately two million man-hours were required to produce the research from which these volumes are derived.

The Administrative Office wishes to express its appreciation to the Project personnel for their confidence and loyalty; to the University as a whole for its support and cooperation; to the many Rochester industries and businesses for the materials supplied and the services rendered; to the Area Engineer's Office for its aid in facilitating the conduct of the program; to Dr. Ellice McDonald, Director of The Biochemical Research Foundation of the Franklin Institute, at Newark, Del., for his cooperation in coordinating the research under his contract with that of Rochester.

Andrew H. Dowdy, M.D.
Professor of Radiology and
Director of the University of
Rochester Project

April, 1949
Rochester, N.Y.

VOLUME EDITOR'S PREFACE

This volume reports the comprehensive experimental studies carried out by the Division of Pharmacology of the Manhattan Department of the University of Rochester under a contract with the Manhattan District.

The material is divided into two parts. Part I deals with the Pharmacology and Toxicology of Uranium Compounds, a subject that received the major attention. The work on the exposure of animals for periods of one year to the inhalation of air containing certain uranium compounds is nearing completion and will be published at a later date.

Part II is concerned with some observations on the toxic action of fluorine and hydrogen fluoride.

The preparation of this volume was authorized early in January 1946 with the request that the manuscripts be completed by July 1, 1946. The fact that the manuscript was completed on the specified date is a credit to all those who took part in this work. Many of the wartime personnel had already left the project. Hence, the writing of the reports had to be done by the remaining personnel. These handicaps account for some imperfections in form of presentation, but do not detract from the value of the scientific contribution.

It is fair to say that the study of the toxicology of uranium compounds herein described represents the most comprehensive experimental investigation of an industrial poison ever carried out by any group of scientific workers in such a short time.

From the standpoint of industrial toxicology, the present investigation has emphasized the great need of new methods for the study of the relationship between particle size of toxic atmospheric dust and the rate and degree of absorption of the toxic material from the respiratory tract. A promising beginning has been made in this field.

The observations on the mechanism of action of uranium on the kidney may be regarded as fundamental contributions (1) to renal pathology and (2) to the understanding of acquired tolerance to a toxic chemical agent.

Carl Voegtlin

April, 1949
Rochester, N.Y.

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The Manhattan Project Technical Section of the National Nuclear Energy Series is intended to be a comprehensive account of the scientific and technical achievements of the United States program for the development of atomic energy. It is not intended to be a detailed documentary record of the making of any inventions that happen to be mentioned in it. Therefore, the dates used in the Series should be regarded as a general temporal frame of reference, rather than as establishing dates of conception of inventions, of their reduction to practice, or of occasions of first use. While a reasonable effort has been made to assign credit fairly in the NNES volumes, this may, in many cases, be given to a group identified by the name of its leader rather than to an individual who was an actual inventor.

HISTORICAL FOREWORD

By Harold C. Hodge

The first days of the Medical Section of the Manhattan Project brought out the immediate need of a toxicological guide for the safety of those handling uranium in laboratories and in plants. How much uranium could be taken into the lungs daily without sericus sequelae? What if dirty hands put milligrams of a uranium salt into an incautious mouth at lunch hour? Would there be trouble, and where? Would drops of a uranium solution splashed onto bare hands and arms cause poisoning by skin absorption? Of the numerous uranium compounds, which were more dangerous and exactly how poisonous was each? The questions were many and the answers few.

There was no time to wait for months, or even for weeks, while the accepted laboratory tests established the toxicological facts. Production had to proceed with no delays. Some working rule had to be adopted pro tem. The toxic heavy metal, lead, had been carefully studied both in animals which had been experimentally exposed and in workers who had been incidentally exposed to its compounds. A maximum daily exposure to 1,500 μg of lead had been proposed and widely accepted as a workable compromise between safety and engineering practice. The average man doing physical labor breathes about 10 cu m of air daily; therefore the maximum allowable concentration of lead permitted in factory air was 150 μg/cu m. The ventilation engineers were able to keep air contamination below this amount, and few persons are made ill by such a minute intake.

No such voluminous literature was available on the toxicity of uranium. A number of papers about the turn of the century had reported unsuccessful attempts to treat human diabetes by uranium salts; unfortunately, the studies of the patients' responses were not carefully done. A specific injury to kidney had been repeatedly and minutely described in various animal experiments. Since milligram doses produced detectable kidney damage in rabbits and dogs, uranium compounds seemed fraught with danger to industrial workers who must handle pounds. Uranium is radioactive; this hazard was also considered but seemed less important for acute exposures than the chemical toxicity. In factory operations the inhalation hazard is considered the most insidious and the most important. No data at all were available on the inhalation toxicity of uranium compounds.

It was imperative that some figure be selected as a tentative maximum allowable concentration for uranium in factory air. The Medical Section selected (wisely as will be seen) the same amount, namely, 150 μg/cu m, which had proved feasible in the case of lead. There was no thought that lead and uranium were alike in their toxic effects or in the way they were handled by the body. The air concentration chosen could be achieved in the lead industry. It should be possible to achieve it in the uranium industry. As far as injury was concerned, a thorough program of health examinations and checkups was started to detect evidences of dangerous exposures.

About this time the first studies in uranium toxicology were initiated.

1. INHALATION HAZARD

It is generally conceded that a major industrial hazard in the handling of inorganic chemicals is the inhalation hazard. The processes of manufacturing are usually designed to obviate high-grade exposures via either the skin or gastrointestinal canal. The dust hazard, however, is serious. In the first place, it is frequently insidious, i.e., the exposed individual takes in a surprisingly large amount without being aware of a high-grade exposure; in the second place, absorption of very soluble materials through the respiratory tract has a unique rapidity which may even approach that of intravenous injection. Consequently, when the problem of determining the toxicities of the various uranium and fluorine compounds was presented in May 1943, the dust hazard was given the highest priority, and the major program was built around the study of animals exposed to atmospheres containing controlled concentrations of the toxic materials.

2. TOXICOLOGY PROGRAM

2.1 Basic Problems. With a working basis established (150 μg of uranium element per cubic meter), the industrial aspects of the project could go forward while biological studies characterized uranium poisoning and determined the relative toxicities of the various compounds. Four basic problems were outlined:

(a) Safety Standards. Development of safety standards for the control of health hazards due to contamination of factory air with uranium, fluorine, and other toxic materials requires:

1. Acute and chronic exposure of various animal species to air containing different concentrations of the toxic materials in order to correlate concentration and toxicity.

2. Construction of exposure chambers and engineering control of the concentration of noxious agents in the chamber air.

3. Elaboration of suitable analytical methods for the quantitative estimation of the various toxic materials, use of such methods in experiments on animals and in the quantitative estimation of toxic material in factory air and in urine samples of workers.

4. Ingestion experiments on animals to determine what part gastrointestinal absorption may play in the toxicity following exposure of animals to contaminated air; intratracheal administration to determine the toxicity by applying the materials directly to the lower respiratory tract.

5. Special studies to determine the local and systemic toxic effects following the application of toxic materials to the skin and eyes of animals.

6. Experiments on the relation between rate of excretion and rate of storage of uranium in the tissues of the body, using the fluorophotometric method, such data to serve as a basis for the interpretation of results of analyses of spot samples of workers' urine.

(b) Protective Devices. Testing of masks and respirators for the emergency use of plant personnel.

(c) Approximate Toxicity Determinations. Tests on new special materials of unknown toxicity (for the guidance of plant personnel).

(d) Provision of Methods for the Recognition and Control of Poisoning. Mechanism Studies. Three principal topics were as follows: kidney function as influenced by uranium compounds; search for a nontoxic complex that will promote urinary excretion and prevent storage of uranium in the body; determination of the degree of acquired tolerance following repeated small doses of uranium.

Field Work. To provide techniques and personnel for collaborative tests assisting plant medical supervisors.

2.2 Preparation. The aim of all this work was to secure information that would eliminate, in so far as possible, hazards to the health of workers handling the toxic materials in plants.

Initially, such studies were to be relatively short (30 days) exposures to find the acute poisonous effects and to determine, if possible, dust concentrations that would not produce toxic effects. On the basis of such information chronic (1 year or longer) exposures were to be undertaken to determine (1) the effects of chronic poisoning, (2) the amounts that could be tolerated by inhalation for a long period with no evidence of damage, (3) the amounts in the atmosphere that would produce some evidences of toxic effect in some animals, i.e., the border zone between "safe" concentrations and toxic concentrations.

Inhalation toxicity studies required intricate machines and apparatus for the production, measurement, and control of dust concentrations. Following visits to the industrial hygiene laboratories of the Dow Chemical Company of Midland, Mich.; the Kettering Laboratories of the University of Cincinnati, Cincinnati, Ohio; the Bureau of Mines in Pittsburgh, Pa.; and the National Institute of Health in Bethesda, Md.,* plans were made and materials were ordered for the construction of the first exposure chamber. A search for technical personnel began. Problems of priorities and lack of materials for construction appeared.

2.3 Relation of Inhalation to Oral Toxicity. Under the conditions selected, it was impossible to distinguish a "pure" lung exposure. Most of the animals were put into exposure chambers unprotected so that there was a possibility of percutaneous exposures and a more important probable exposure, the oral one. The animals licked their fur more or less and ingested a certain amount of toxic material in this way. Furthermore, any particulate material that is taken into the lung may be deposited in those parts of the respiratory tract lined by ciliated epithelium. The cilia have the function of bearing up through the trachea any particulate material that falls thereon. Thus a sizeable fraction of that material deposited in the lung may ultimately arrive in the gastrointestinal tract. This tract also may be the route by which a part of other particulate material caught in the upper respiratory and nasal passages is removed. Of course, the solubility of the particulate material influences this picture to a marked degree. Highly soluble substances would probably be absorbed through the mucose of the upper respiratory tract and the epithelium of the lung. It is apparent that some knowledge of the oral toxicity of the various substances is necessary for the interpretation of the lung-exposure data.

Preliminary information on oral toxicity can be acquired very rapidly by using colonies of rats. Such a program was promptly set up, and by the end of the summer of 1943 a listing had been made of a number of uranium compounds grouped roughly according to their oral toxicities.

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*It is a pleasure to acknowledge the courtesies of Don D. Irish and staff of the Dow Chemical Company; F. F. Treon, R. A. Kehoe, and Edward Largent of the Kettering Laboratories; A. N. Sayre, H. H. Schrenk, and coworkers at the Bureau of Mines; and Lawrence T. Fairhall and associates at the National Institute of Health.

2.4 Toxicological Priorities. Based on this information, with the additional data furnished by Albert Tannenbaum from comparable studies on mice, a listing of the approximate toxicities of these compounds was made in September 1943 and amended in March 1944. In the choice of toxicological priorities, two factors were considered: (1) the toxicity and (2) the number of persons who had real or potential exposures to each compound. When these two ratings were combined the toxicological priorities were ordered as shown in Table 1. This list of priorities was to serve as a guide in planning the program of inhalation exposures.

Table 1—Toxicological Priorities

Compounds	Rating from number of persons exposed	Rating from relative toxicity
Uranium:		
1. $UO_2F_2(UF_6)$	1	1
2. $UO_2(NO_3)_2$	2	2
3. Uranium fumes	2	3
4. UCl_4	4	2
$UOCl_2$	4	2
5. UO_3	3	
6. UO_4	4	
7. UF_4	3	4
8. U_3O_8	2	5
9. UO_2	3	5
10. $Na_2U_2O_7$	4	
11. $(NH_4)_2U_2O_7$	4	
Non-uranium:		
1. F_2		2
2. HF_2		

3. PERSONNEL

A large number of people have been associated with the project and have given wholeheartedly of their ideas and energies. It seems impossible to give individual mention to each of those who contributed in an outstanding fashion to the success of this project. Some notion of the degree of responsibility that various people have assumed may be gained from the designations in the list of personnel given in Appendix

III, following Part II. The term of service is indicated by the dates placed immediately after each name.

The Pharmacology Division. Here is included Carl Voegtlin, who served as Consultant and as Chief Toxicologist, and whose guidance was obtained at every step. It is not too much to say that, without his mature knowledge of pharmacology and his experience as an administrator, the work reported in this volume would have gone haltingly indeed. His was a major contribution.

Here also are listed consultants whose expert advice was obtained in the special fields indicated. J. F. Treon, with his experience in similar laboratory studies involving exposure chambers, gave especially helpful advice. H. H. Schrenk and S. J. Pearce guided, both technically and strategically, the experiments that were carried out on respiratory protective devices. H. S. Gardner assisted with the chemical engineering problems. D. R. Goddard advised on certain metabolic problems and critically reviewed certain studies.

(a) Toxicology. This section, under the leadership of Herbert E. Stokinger, was responsible for the inhalation studies of toxicology.

(b) Pharmacology. This section, under Frances Haven, was responsible for oral toxicological studies, skin and eye toxicity studies, distribution and excretion studies, and a number of biochemical problems.

(c) Mechanism. Under the leadership of Alexander Dounce, this section undertook the study of the effect of uranium on enzymes and protein, the physicochemical behavior of uranium in solution, and the role of the kidney in uranium poisoning.

(d) Engineering. Under Capt. Geoffrey Goring and Sgt. Neil Murphy, the engineering section designed and built the multiplicity of devices used in connection with the exposure chambers.

(e) Analytical. Under the direction of John Flagg, the analytical section had the responsibility of conducting research activities on analytical methods. All routine biochemical determinations and the routine fluoride determinations were carried out in this program.

(f) Collaborating Divisions from the Manhattan Project of the University of Rochester. A number of persons are listed under collaborating divisions. Most of these divisions performed functions and services beyond their contacts with the Pharmacology Division. A few persons are mentioned in each of the collaborating divisions in an effort to give at least a small acknowledgment of the important role played by their work (see Appendix III).

4. THE VALUE OF ANIMAL EXPERIMENTATION

One of the most discussed questions when toxicologists gather is:

What is the usefulness of animal toxicity data? There was a general agreement that, if animal lives can preserve and safeguard the health of the laboratory and industrial personnel engaged in the work on atomic energy, the animal lives were expendable. It is worth while to review briefly some of the many arguments as to how animal studies can serve to safeguard the health of the scientists and industrial personnel.

4.1 How Toxic Is a Compound? There is no fixed constant of toxicity (corresponding to a boiling point or a molecular weight) that may be used to characterize the toxicity of a material. It is impossible to state exactly what the toxicity is even for a single animal because of the wide variations in responses that any normal organism goes through from time to time. Furthermore, individual susceptibilities show such a variation that the measurement of toxicity is almost a statistical matter. Extremely minute doses that would fail to injure most animals will produce visible poisoning in the highly susceptible individual. As large numbers of animals as feasible are always employed in an effort to minimize, or even to take advantage of, this variation.

The not too satisfactory, but widely accepted, basis for expressing the toxicity is the collection of as much information about as many species as seems practical. Man would be expected to form a part of the series if all species were arranged from the most susceptible to the most resistant. There are no rules of order about the susceptibility of a given species. The rat, for example, may be highly susceptible to one compound, such as UF_4, and highly resistant to another, such as UO_2F_2, whereas the mouse exactly reverses these positions. Nevertheless, these generalities can be offered:

1. Somewhere in the line-up of susceptibility, man must stand.
2. It has generally been the case that a given compound is highly toxic to many species, moderately toxic to many species, or relatively nontoxic to many species.

Thus, by obtaining the relative toxicity of one of the uranium compounds in several species, the order of magnitude of the toxicity in man may be postulated.

One further complication must be mentioned. There are certain compounds, the most notorious of which is crystalline silicon dioxide, or quartz, in which the toxic effect of the molecular aggregation is entirely disproportional to the expected toxicity as compared with other more or less related chemical molecules. The possibility must be kept in mind that a given compound may have a unique molecular toxicity. It has even been observed that certain specially toxic molecules may have no effect or little effect on one species and have a characteristic toxic action on others. For example, triorthocresyl phosphate has a unique toxicity unlike closely related chemical bodies. This compound produces a peculiar and characteristic paralysis in calves, cats, and dogs but seems to have no similar effect on rats and mice.

In general, no better procedure has been suggested than obtaining the limits of toxic effect as related to dosage on a number of species and judging that the effects on man would be produced by doses of the same order of magnitude.

4.2 Acute Toxic Effects. The description of the course of acute poisoning and the estimation of the doses that produce acute symptoms including death are two of the toxic properties usually first studied. In observing different species the toxicologist looks for different qualitative effects. The question arises: Does the material always involve or damage certain organs or systems in every species? It is desirable to fix the course and duration of the poisoning; therefore the symptoms are recorded in the order of their appearance. Studies designed to determine the structural effects of the poison and also to determine the functional derangements are carried out. The object of this study is the delineation of an entity described as "acute" poisoning.

4.3 Chronic Toxic Effects. By the simple procedure of decreasing the amount of a single dose of a toxic material, a point is presently reached where no detectable injury is produced. The long-term administration of such amounts of certain substances produces a state called "chronic intoxication." The first question concerning chronic toxicity is: In what way does it differ from acute poisoning? The next problem is to discover the time relationships of dosage to the appearance of signs of poisoning. In practice, some regimen of administration can be found under which exposures day by day to known amounts of toxic agent produce in time evidence of poisoning. This might be called a "positive" response.

It is axiomatic that the dosage of any toxic agent may be reduced to such a low point that the administration at this level for the lifetime of the animal would fail to show any evidence whatsoever of injury as a result of this exposure. This might be called a "negative" response. Some place in between the negative zone and the positive zone is an area of plus or minus injury in which some criteria of damage might be discovered to be positive, whereas others would remain consistently negative. If this plus or minus zone could be located approximately in the case of uranium poisoning, then the amounts of uranium dusts that could be permitted in the atmosphere of workrooms would be estimated with known factors of safety. The studies of chronic inhalation toxicity were set up to discover, if possible, (1) a certainly safe toxicologically noneffective concentration of uranium dusts in the air and (2) the approximate location of the plus and minus zone wherein some animals would show some evidences of injury, but wherein all animals would not show a uniform injury response to any single criterion. In this wise, some notion of the toxic limit of exposures could be established.

Of the phenomena that appear in chronic-toxicity studies one of the most interesting is the development of a tolerance. This is a common phenomenon, well illustrated by the average tobacco smoker who tolerates with only pleasurable feelings exposures to nicotine that in the novice would prove noxious indeed. The discovery of the existence of tolerance and the elucidation of the mechanism by which tolerance to uranium is produced are problems susceptible to study by animal experimentation.

Another important effect peculiar to chronic administration is the cumulative action of certain materials. Among the common poisons, three — lead, arsenic, and fluoride — are famous for their cumulative actions. An unusual angle to the possibility of cumulative action of uranium is whether radioactivity might be a factor.

As in the acute toxic reaction, it is necessary to observe structural as well as functional effects of chronic administration. In this regard, there is an interesting question of the relative life span of rodents or of dogs as compared with that of man. There is a time-honored concept that, since two or three years is the life span of a rat, one year in the life of the rat must roughly equal thirty or forty years in the life of a man. Consequently, an exposure of a rat for a month or two would give effects such as might be observed in man following several years' exposure. It is possible that in certain biological systems such a comparison might be made. It seems equally probable that, for responses that depend on the fundamental reactivity of protoplasm, a month's exposure would give the same responses whether the protoplasm was that of a rat or that of man. Certainly, for materials to which man is to be exposed for periods of years, it is reasonable to study the effects in animals exposed for their lifetimes.

4.4 Factors Influencing Toxicity. Gelman* in 1936 pointed out what he called the three fundamental principles of modern toxicology: (1) the action of a poison depends both on the chemical structure and the physical state of the material at the time of its poisonous action; (2) the qualitative and quantitative responses to the poison are determined by the route of penetration; (3) the toxicity does not depend solely on the toxicity of the elements that make up the molecule. It will be seen in the presentation of the toxicological data in later sections that each of these principles has been considered. Uranium has been studied in various chemical combinations in various physical states. Animals have been exposed via the mouth, eyes, lungs, skin, peritoneal cavity, subcutaneous tissue, and by intravenous injection. The principal toxic response was in each case attributed to the uranium.

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*I. Gelman, J. Ind. Hyg., 18: 371 (1936)

4.5 The Mechanism of Toxic Effects. One of the first correlations attempted with the animal studies is to establish some measurable physical, physiological, or chemical effect that is an index of the degree of injury. There is general agreement on the desirability of establishing the concentration of a poison in the blood stream as a measure of the degree of exposure, and thus of the degree of injury. Once these relationships are established, a simple analysis of a blood sample can tell much about the history and prognosis of the poisoning. Unfortunately, as will be shown, this convenient procedure is not applicable to uranium poisoning.

Another important piece of information about any poison is its state in the body. There is a tendency to concentrate on what the poison does to the body to the exclusion of studying what the body does to the poison. Using experimental animals, the distribution of uranium in the various tissues of the body, the routes and rates of excretion, and the speed and mechanism of storage have all been studied. These values permit deductions and predictions to be made about the treatment of human exposures to uranium compounds. Something of the metabolic pathways of uranium have been discovered, and a reasonably satisfactory story of the handling of uranium by the body has been worked out.

In these days when knowledge of enzyme chemistry is growing so rapidly, it is inevitable that the question should arise: Does uranium produce its poisonous action by virtue of its ability to inactivate or otherwise alter some enzyme system? From the tissues of poisoned animals, sections (thin slices) and extracts of various sorts have been examined by special techniques to determine what circumstances, especially what concentrations of uranium, destroy the normal activity of the enzyme. These data can then be correlated with concentrations of uranium found in poisoned animals.

4.6 Early Detection of Poisoning. If a human population suffers an unavoidable exposure to a toxic material, one of the desiderata is an extremely sensitive method for the detection of the earliest signs of injury. In the experimental animal, various diagnostic procedures may be tested, and, after a given exposure, the degrees of change can be correlated with the severity of injury. In such a search the blood count and urine analysis have a peculiar usefulness. Plant personnel have by now become more or less accustomed to occasional routine blood counts. In many plants it is easy to obtain urine samples, when desired, from cooperative employees. Thus, methods of detecting early changes in the blood or in the urine not only serve as criteria for following the acute and chronic toxic effects in exposed animals but also have a special value as possible aids to the medical supervision of plant employees.

4.7 Restriction of Survey Studies on Human Beings. The importance of good animal experimentation is emphasized by the fact that experimental groups of human subjects are unavailable under any circumstances. On several occasions when an especially promising and delicate method for detecting early signs of uranium poisoning was perfected in the laboratory, volunteers from among the laboratory workers would come forward asking that they be permitted to take small amounts of uranium and apply these tests to themselves. Such exposures were never allowed.

There is one group of human beings, the industrial personnel handling the uranium compounds, in which there was a necessary and unavoidable exposure. This exposure was always maintained at the least possible level, and frequent medical checkups protected the health of these workers. The medical findings were so consistently negative that most of the basic information on the health practices for employees in uranium plants would not be known if experimental animals had not been used.

4.8 Prophylaxis. The practices of vaccination and immunization against certain erstwhile dangerous infections have been so successful that inevitably some reliable protection was sought against uranium poisoning. The questions were: Is such protection possible? If so, what is the mechanism by which the body achieves this degree of resistance? Are there any practical methods that would improve the safety of working conditions? Although to date these investigations have not been fruitful in the prophylaxis of uranium poisoning, the diligence of inquiry is plainly visible in the following pages.

4.9 Treatment of Uranium Poisoning. Accidents do happen, and it seemed inescapable that now and then some series of misfortunes would provide a sufficient exposure to produce some evidence of poisoning. Attempts were therefore made to find any agents that would alleviate or improve the condition of animals either acutely poisoned or undergoing a long-term chronic exposure. Studies of the mechanism of poisoning shed light on the methods by which treatment might be instituted. A number of agents were examined and evaluated as to the efficacy and danger of the antidote in comparison with the danger of the poisoning.

4.10 Medical Supervision. In Chap. 16, Capt. Joe Howland has broadly outlined the thorough medical supervision instituted under the direction of Col. Stafford L. Warren. This laboratory collaborated by making analyses as requested for the uranium content of urine and for the fluoride content of urine of industrial personnel. A beginning in the broad field of industrial hygiene was made by studies of the dust concentration and particle size in the air of certain workrooms. The principal reason for mentioning the medical care at this point is to point out that toxicology is not in any sense a substitute for medical supervision. It can be an aid to medical supervision.

4.11 Maximum Allowable Concentration. One of the most discussed concepts in industrial hygiene is that of the maximum allowable concentration. This is a number, e.g., 150 μg of uranium per cubic meter of air, that serves as a bench mark by which engineers compute ventilation requirements. Under wartime conditions, emergency measures were necessary in order that production should proceed at maximum speed, and exposures were occasionally justified which in peacetime with adequate ventilation, housekeeping, and control would not be tolerated. In wartime, in order to operate a plant it might be necessary to require a man to be present in a room that contained a higher concentration of uranium dust than was certainly safe physiologically to experimental animals. Under peacetime conditions of operation, such an exposure would be unthought of. In the case of breakdowns, leaks, or accidents, unavoidable exposures might occur in peacetime or in wartime, but the only reasonable rule is to maintain the air of workrooms at such a degree of purity that no experimental animal would reveal any signs of poisoning from its inhalation. That the procedures actually followed were justified is proved by the fact none of the workmen ever showed, even after years of exposure, a toxic effect that could be attributed unequivocably and solely to uranium dust in the workroom atmosphere.

5. SECURITY REGULATIONS

This record would not be sufficiently complete if mention of the psychological factors were not made. Almost without exception, the members of the scientific and technical staff came from academic surroundings. As students they had enjoyed the practice of open and frequent discussion; as teachers, they had been paid to tell "what they knew." Their success depended on their ability to impart knowledge. As researchers their right of publication was unquestioned, in fact, expected. Sometimes spontaneously a coincidence of interest had drawn a few men into small productive units or research teams, but more often each depended on his own thoughts, the laboratory, and the library.

It was a shock to be forced overnight to abandon the habits of years, to deal predominantly with "classified" information, to seek "clearance" before opening a technical discussion with a colleague. Common laboratory chemicals suddenly became "secret." For example, HF, which sits in a wax bottle in nearly every chemical storeroom, could be spoken of or written about only in code. A thick book of rules and regulations prescribed the details, and what must be done in case of an infraction. Security regulations, more than any other single fact, brought home the secret nature of the project.

War research, as it developed on this project, was an entirely different form of activity from the customary academic medical research. In its less esoteric sections, the work was "production"—highly routine, mechanical, hard physical work. On the more intellectual problems there were groups of specialists with their assistants who pooled all ideas with no considerations of "priority of publication" or of personal gain. The rapidly expanding groups brought together coworkers who exhibited mutual tolerance and acceptance of relationships that in peacetime would never have existed. Too much praise cannot be given the men who by their hearty, unstinting cooperation produced the desired health information. Their fine spirit made the project go.

INTRODUCTION

By Harold C. Hodge

Part I. The Pharmacology and Toxicology of Uranium Compounds

1. CHEMISTRY OF URANIUM COMPOUNDS

The chemistry of uranium compounds in its biologically important aspects has been presented under three headings: (1) the compounds involved have been described briefly; (2) the complexing behavior of uranium in solutions has been described in some detail; (3) the oxidation-reduction behavior of uranium has been studied, especially under conditions bearing some relationship to physiological conditions.

1.1 Chemical Compounds. Two principal ores of uranium are pitchblende (U_3O_8) and carnotite (a potassium-uranium-vanadium mineral).

The valence forms of uranium are 3, 4, 5, and 6 in the dry state, and 6 and 4 in solution.

A number of oxides of uranium are known: U_3O_8, the black oxide, a mixture of U_6 and U_4; UO_4, the pale-yellow peroxide; UO_3, frequently called the "yellow" oxide; and UO_2, referred to as the "brown" oxide.

A number of fluorides are known, of which the most interesting is UF_6, a gas. The compound UF_4 is known commercially as "green salt"; as commercially handled it usually contains 1 per cent or more of uranyl fluoride, UO_2F_2, which is a pale-yellow material. Two black fluorides, UF_3 and U_2F_9, are known; another fluoride, UF_5, is white.

Several chlorides are known. Uranium tetrachloride, UCl_4, occurs in the form of green crystals; UCl_5 and UCl_3 are also green compounds; and UCl_6 is yellow.

Several carbides are known, among which UC, U_2C_3, and UC_2 are to be noted in particular. Two nitrides have been described, corresponding to the formulas UN and $UN_{1.5-2}$. The hydrides of uranium are useful in certain processes, especially UH_4.

Two uranyl salts that have widespread use are uranyl nitrate, which consists of yellow crystals whose solubility in organic solvents is well known, and uranyl acetate, which occurs as yellow prisms.

1.2 Complex Formation. One of the outstanding properties of uranium in aqueous solution is the formation of anionic complexes. These have been studied by experiments using electrophoresis, the polarograph, the spectrophotometer, various color reactions, titration procedures, and protein-precipitation procedures. As a result of the electrophoretic studies, the various complexers may be listed in three groups according to their strength in complexing U_6. Each succeeding group of the following three represents more powerful complexers for U_6 in solution:

Group I	Group II	Group III
Acetate	Pyruvate	Malate
Lactate	Glyceryl phosphate	Citrate
Phosphate	Proteinate	Bicarbonate
Maleate		Carbonate

The pH has an effect on the degree of complexing only in so far as pH determines the concentration of the anion in the buffer solution. Hexavalent uranium complexes only with the anion at physiological pH's. Apparently SH groups are not involved in complexing U_6.

Studies of the absorption curves of uranyl solutions have been important in distinguishing weak from strong complexers. The ferrocyanide color reaction with the uranyl ion has been especially useful.

In titration experiments with various proteins, e.g., with albumin, it has been concluded that U_6 complexes with the carboxyl groups of the protein. Hexavalent uranium tends to distribute itself between the protein and bicarbonate complexes in the plasma. Ultrafiltration studies have shown that the tendency of U_6 to complex with bicarbonate increases with the concentration of this ion. In animals having a normal CO_2-combining capacity of the blood, U_6 is presumed to be about equally distributed between protein complex and bicarbonate complex. In the blood, complexes with albumin, globulin, and fibrinogen have been found.

The strong tendency of a protein to form complexes with U_6 has been used in the procedure for isolating U_6 in analytical work in which, for example, separations are made from calcium and phosphate. Protein is introduced, and then the U_6-protein complex is precipitated by heat denaturation of the protein component.

Tetravalent uranium can form complexes with acetate, citrate, and bicarbonate. Contrary to the case with U_6, it appears that SH groups have some tendency to complex U_4. An important difference between U_6 and U_4 is that U_6, freshly precipitated as oxide, will dissolve in the presence of strong complexers, whereas U_4, freshly precipitated as oxide, will not. This raises the question as to whether an appreciable amount of the U_4 in the plasma of animals that have received U_4 intravenously may exist in the form of an oxide, probably stabilized as a colloid.

1.3 Oxidation-Reduction Studies. The U_6,U_4 system constitutes a somewhat sluggish oxidation-reduction system. The kinetics of the oxidation-reduction reactions at the platinum electrode or at the dropping-mercury electrode are determined by the rate of the formation-dismutation reaction in which U_5 is formed according to the following generalized equation:

$$U_6 + U_4 \rightleftharpoons 2U_5$$

At equilibrium there is very little U_5 present, so that if U_5 is dissolved in water it is unstable and immediately forms U_6 plus U_4. Trivalent uranium is also unstable in solution and quickly changes to U_4 through oxidation by dissolved oxygen or, more slowly, through oxidation by water itself.

The oxidation potential of the U_6,U_4 system is dependent upon pH, in the pH range of 2 to 7. In general, increasing the pH tends to make reduction of U_6 to U_4 more difficult thermodynamically. The reduction of U_6 to U_4, or the reverse process of oxidation, can be broken down into two steps consisting of the U_6,U_5 step and the U_5,U_4 step. The first step is not dependent upon pH, while the second step (U_5,U_4) is dependent upon pH. It is this second step that makes the over-all U_6,U_4 equilibrium dependent upon pH.

General equations that describe the oxidation-reduction equilibria of the U_6,U_4 system at the platinum electrode have been written, corresponding to the system in the absence of complexers or in the presence of acetate in two different ranges of concentration. In the latter case, when the ionized acetate concentration is not greater than 0.05M and the total uranium concentration is not greater than 0.002M, it has been found from polarographic studies that U_6 is complexed with two acetates, U_5 with zero acetates, and U_4 with two acetates. If the acetate concentration is greater than 0.05M, the U_6 becomes complexed with three acetates, the U_5 with one acetate, and the U_4 remains complexed with two acetates.

Work has been carried out on the U_6,U_4 system in acetate buffer partly to serve as a model study of uranium in the presence of complexers. Considerable detailed material pertaining to this system has been presented, including a table of standard potentials for the U_6,U_4 system and a table of the dissociation constants for various uranium acetate complexes, as calculated from polarographic data.

In regard to the level of oxidation of uranium in the body, U_6 appears to be the most stable form. Trivalent uranium and U_5 cannot be stable in appreciable concentrations. Under the conditions believed to exist inside the living cell, a partial reduction of U_6 to U_4 is possible, but, since there is little evidence that U_6 penetrates into cells in significant amounts, the reduction of appreciable amounts of U_6 in cells is considered to be unlikely. Reduction of U_6 in plasma does not appear to take place, and its reduction in extracellular fluids is unlikely.

Tetravalent uranium in aqueous solution is readily oxidized by atmospheric oxygen, although at a moderately slow rate, and an appreciable oxidation of U_4 probably occurs in vivo, particularly in the blood stream. Since the rate of oxidation of U_4 by oxygen is retarded by substances present in the body, such as protein and SH groups, its rate of oxidation in vivo must be even slower than in aqueous solution in vitro. Distribution studies have confirmed the persistence of a considerable portion of injected U_4 in the body for a considerable length of time, which may extend from a number of hours to a number of days.

2. ANALYTICAL METHODS

A number of analytical methods have been employed, in some cases exactly as has been described in the literature. In most cases, modifications have been worked out that have improved the method as applied to the problems at hand. The methods are described in detail to enable reproductions of the techniques.

In Table 1 are listed in very brief fashion the various methods for uranium determination, the amounts of uranium for which the method is suited, an estimated error of the method, and a rough approximation of the time required per analysis. The methods are as follows: volumetric, 0.5 to 10 mg; ferrocyanide, 80 μg and more; polarographic, 50 μg and up; spectrochemical, 0.1 to 50 μg/g; fluorophotometric, 0.002 to 1,000 μg.

The errors range from about 1 to 17 per cent. In the volumetric method, the error is 1 to 3 per cent; in the polarographic method, about 3 per cent; in the ferrocyanide method, about 5 per cent; in the spectrochemical, 10 to 17 per cent; and in the fluorophotometric, 4 to 7 per cent. In each case, the error is ± the number given.

The volumetric method and the ferrocyanide method are fairly rapid; 30 to 45 min is required. The polarographic method requires about an hour, the spectrochemical and fluorophotometric methods are much more demanding, the former requiring about a half day and the latter about a day. It should be noted, however, that many analyses may be performed by the latter methods in not much longer than is required for a single determination.

Table 1 —Summary of the Methods of Uranium and Fluorine Determination

Methods	Amount	Error, %	Time required
	Uranium Determination		
Ferrocyanide	80 μg up	±5	30 – 45 min
Fluorophotometric:	0.002 μg up		
Pure solution	0.01 – 10 μg	±4	About 1 day
Tissues	0.5 – 1,000 μg	±7	About 1 day
Polarographic	50 μg up	±3	1 hr
Spectrochemical:			
Pure solution	10 μg	±10	About ½ day
With RbCl	0.5 μg	±10	About ½ day
With $K_2S_2O_7$	0.1 μg	±10	About ½ day
Soft tissues	0.2 – 1.0 μg/g	±17	About ½ day
Bone	1.5 – 50 μg/g		
Volumetric	0.5 – 10 mg	±1 – 3	30 min
	Fluorine Determination		
Long method	3 – 30 μg	±5	About 1 day
Short method	1 – 100 μg	±1 – 6	About ½ day
Enzymatic	0.0 – 0.5 ppm	±5	1 to 2 hr
With Zr	10 ppm		
Polarographic	1 μg/ml in 30 ml	±7	1 hr
Ferrisal	5 mg	±10	1 hr

In the same tabulation are given several methods for fluorine determinations. The same information is listed as was given for the uranium methods. The "long" and "short" methods, which are modifications of the Willard and Winter procedures,* showed from 1 to 100 μg. The enzymatic and polarographic methods showed amounts of the order of 1 μg/ml and even less; the total samples were from 1 to 30 μg. The "ferrisal" method error, about 10 per cent, is a little greater than the error for the other methods.

The polarographic and the ferrisal procedures require about an hour; the enzymatic method can be carried out in 1 to 2 hr. The long and short methods require about 1 day (for one or several samples). In each case, most of the time is spent in ashing and distillation. Differences in time are in the final titration.

3. STATISTICAL METHODS

The statistical work in connection with the data gathered on the pharmacology program has been designed to perform three functions:

1. To organize and present the experimentally determined numbers

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*H. H. Willard and O. B. Winter, Anal. Chem., 5: 7 (1933).

in a compact, convenient form; to summarize such groups of data by locating a measure of the central tendency and by presenting some measure of variability.

2. To discover and describe quantitatively the correlation between variables studied experimentally.

3. To find, when experimental work has been finished, whether the conditions of the exposure were such as to have altered the health of the animals. The significance of differences between control and experimental animals is expressed in terms of a probability value indicating how often by chance such a difference would occur.

4. PATHOLOGICAL ANATOMY OF URANIUM POISONING

The groups of animals on which the descriptions of pathological changes and the interpretations of these changes are based are carefully delineated.

4.1 Renal Damage. Four grades of severity of renal damage are described in rat kidney, ranging from mild to very severe.

Grade I, Mild. The first evidences of degenerative changes in the tubular epithelium are visible 3 or 4 days after the initial exposure. These changes persist for 10 to 14 days. In the meantime, active regeneration is apparent, and by the thirtieth day the tissue in general appears normal. Changes are observed only in the proximal convoluted tubule.

Grade II, Moderate. The changes in this classification are more extensive and less transient than those of Grade I. The first changes are seen in 1 to 2 days and are characterized by tubular degeneration and necrosis. Debris-filled tubules are observed. In about 2 weeks the active regeneration produces a tubular lining that may be typical or atypical in form, the former usually predominating. Some degenerating cells call attention to the fact that the toxic agent is still active. Sometimes changes are found lower in the tubule, perhaps in the loop of Henle. This picture does not become entirely normal by the end of the month of repeated daily exposure by inhalation or continued feeding. In fact, at the end of 2 years, changes are still present, consisting of tubular atrophy, dilation, and distortion.

Grade III, Moderately Severe. The renal damage included in this classification is of greater intensity than the preceding grades and appears early, in the first day. Completely necrotic tubular segments are observed soon after exposure begins. Other kinds of abnormalities may be found, e.g., calcification. In 2 weeks the regenerative process is well advanced, and both typical and atypical epithelial cells are described. Thus, considering the severity of the onset, the amount of repair by the end of 30 days of exposure is remarkable.

Grade IV, Very Severe. From extremely high doses, almost immediate cell destruction is found, e.g., in 1½ to 2 hr. The proximal convoluted tubule is extensively involved. The degeneration is more advanced, more widespread, and may extend farther along the nephron.

Dogs and rabbits exhibit changes very much like those described in rats. The changes in dogs and rabbits are likely to occur following lower doses than are required for comparable changes in rats. The abnormalities in dogs and rabbits are frequently greater at 30 days than in rats. However, the principal qualitative differences between dogs and rats appear in the more chronic stages of the lesion.

4.2 Pathology in Other Tissues. In the digestive tract little was observed except in the intestinal mucosa and submucosa of dogs fed large amounts of uranium compounds. Sometimes hemorrhages were observed in these tissues. Occasionally ulcers were seen in the buccal mucosa. Fatty livers were observed in certain rabbits and dogs severely poisoned. Bone-marrow studies showed no changes that are considered to be of significance from the viewpoint of toxicology. Lung pathology was observed in certain of the soluble compounds at high dust concentration, e.g., UF_6 at 20 mg/cu m.

No changes attributed to uranium were observed in the following tissues: spleen, lymph nodes, gonads, heart, skin, adrenal, thyroid, pancreas, brain, and certain nerves.

4.3 Studies of Kidney Regeneration. Considerable attention was directed to the origin of the regenerated epithelial cells.

In certain experiments other kinds of kidney injury were described. Glomerular changes were infrequent, although, in severe poisoning, abnormalities are sometimes seen in glomeruli. An increase in intertubular stroma follows the injury responses in certain cases.

5. CHARACTERISTICS OF URANIUM POISONING

5.1 Local Effects. Some of the uranium compounds cause irritation when applied to the skin. A few cause intense irritation when applied to the eye. In high concentrations the respiratory tract may be injured by some of the soluble compounds. Ingestion, even of large amounts, failed to produce injury of the gastrointestinal tract in rats. High concentrations produced hemorrhage of the gastrointestinal tract in dogs but probably not by direct local action.

5.2 Systemic Action. Uranium rapidly leaves the blood and is probably deposited in the bone or excreted in variable amounts via the kidneys. Data are offered to show that in oat-fed rabbits, with acid urines, infused with saline, only 7 per cent of the injected dose of U_6 is excreted within 3 hr after the injection. In a non-oat-fed saline-infused rabbit, 20 to 40 per cent of the dose is excreted; and, in a bicarbonate-infused rabbit, as much as 80 per cent of the dose may be excreted in 3 hr. In general, uranium clearance increased with increased pH of urine. The presence of the bicarbonate ion in the urine had an especially striking effect in increasing the urinary excretion of U_6. The uranium removed from the blood by the kidney is either excreted in the urine or deposited in the kidney, so that in the cases just cited kidney retention accounts for any uranium filtered through the glomerulus which does not appear in the urine. The importance of these observations is that they indicate that the urinary uranium content is not a reliable indicator of uranium poisoning.

Renal clearances have given considerable information concerning the site of uranium deposition in the kidney tubule. Glucose, which is reabsorbed almost completely in the first half oi the proximal convolution, undergoes considerable increase in clearance after injection of uranyl salts, indicating some loss of function in this area. Chloride, urea, and amino acids are reabsorbed partly in the proximal convoluted tubule and partly elsewhere; inconstant changes in the renal clearances of these substances, produced by U_6 injection, indicate dispersal of the reabsorptive mechanism. Phenol red and diodrast are excreted by the proximal convoluted tubule; marked decreases in the clearances of these substances after injection of U_6 indicate severe damage to cells in that area. Inulin, creatinine, and xylose appear in the urine in the same concentrations as in the glomerular filtrate; no changes were observed in the clearances of these substances after uranium injection, indicating that uranium has little or no action on the glomeruli.

The urinary ratio of amino acid nitrogen to creatinine was found to be relatively independent of the rate of urine flow. Since the concentration of creatinine in the urine measures the degree of water removal from the glomerular filtrate, an increase in the ratio of amino acid nitrogen to creatinine means a decreased reabsorption of the former substance in relation to the resorption of water. This has been found to be a useful index of the toxic action of uranium for the tubule, as has the removal of phenol red from the blood plasma.

Immediately following administration of uranium, the chloride and inulin clearances were unchanged, whereas the diodrast clearance decreased. Later, all clearances decreased. The latter finding is due perhaps to mechanical obstruction of the tubules by cellular debris and breakdown of the normal barrier to back diffusion from the tubule lumen to the blood.

Urine flow usually increased perhaps twofold, and then slowly decreased. In advanced poisoning, oliguria and anuria developed. Since the chloride clearance increases with urine flow, this fact must be taken into account in interpreting the studies of renal function in poisoned animals. In the inhalation studies, decreases were found in the clearances of inulin and especially of diodrast. Few significant changes in chloride clearances were found.

A number of changes in the concentrations of substances in the blood developed. There was acidosis, the blood NPN and urea nitrogen levels increased, and the glucose tolerance curve was prolonged. The iodine number of the phospholipid fatty acids of the blood increased significantly in the poisoned rats. There were decreases in the plasma levels of prothrombin and fibrinogen, and in the removal of bromsulfalein from plasma.

The urinary excretions of catalase, phosphatase, and protein were found to be increased after intoxication of animals with a variety of poisons. In general, catalase excretion was the most sensitive of these tests as an indicator of tissue destruction in the tubular region.

The excretion of urine increased soon after poisoning with uranium and decreased later on. It might fall to the level of anuria. Urinary pH showed a similar cycle, first increasing and then decreasing. The excretions of various organic acids in the urine were increased after poisoning.

The urinary excretion of NPN substances other than amino acid nitrogen was decreased markedly. The decrease in the excretion of these substances was found to be great enough to explain the azotemia found experimentally. Ammonia excretion increased or decreased, depending probably on the balance between acidosis, with its stimulus to renal formation of ammonia, and parenchymal damage, with its abolition of ammonia formation.

Table 5.12 shows the control values of many parameters.

6. TOXICITY FOLLOWING THE PARENTERAL ADMINISTRATION OF CERTAIN SOLUBLE URANIUM COMPOUNDS

The mortality in rats following the administration of a single acutely poisonous dose of uranium, given either intraperitoneally or intravenously, is an important figure. Estimations have been made of the mortalities after such large doses that many rats failed to survive 24 hr. Since in uranium poisoning the kidney is the site of the most severe injury, uranium produces a high mortality in the week or two following administration of much lower doses. In addition, some studies have been made of the mortality in mice in the experimental periods of 2 to 3 weeks. The intravenous work was limited to exploratory tests.

6.1 LD$_{50}$, 24 Hr. Groups of rats were given various doses of the soluble uranium compounds indicated in Table 2. The mortalities produced by various doses were converted into probits, and the data handled statistically to derive the LD$_{50}$. This dose may be conveniently described as the one that will kill the average rat. Male rats of body weight 50 to 100 g required 305 mg of uranyl nitrate hexahydrate per kilogram to produce the LD$_{50}$. With increasing body weight, and therefore with increasing age, the LD$_{50}$ dropped to approximately one-third this amount. Female rats weighing 200 to 300 g had about the same LD$_{50}$ as 300- to 400-g male rats. The difference in body weights is not important; the controlling factor is that the rats were of about the same age.

Table 2—LD$_{50}$ for Male and Female Rats and Mice

Body weight, g	$UO_2(NO_3)_2.6H_2O$, mg/kg	UO_2F_2, mg/kg	UCl_4, mg/kg
	24-hr Mortality		
Male rats:			
50-100	305		
100-200			434*
200-300	204		
300-400	128	40	297
Female rats:			
200-300	135		190†
Male and female rats:			
50-100		78	335† 615*
150-200		87	
	Mortality in 14-21 Days		
Male rats	2	2.5	5 ± ?
Female rats	1	1	5 ± ?
Male and female mice:			
White	6-8		
C_2H	20-25		

*Five per cent in sodium acetate.

†Ten per cent aqueous solution.

Much smaller amounts of UO_2F_2 than of uranyl nitrate were required to produce the LD$_{50}$. On the other hand, much larger amounts of UCl_4 were required when the UCl_4 was made up in acetate buffer. For 300- to 400-g male rats, the LD$_{50}$'s were of the following magnitudes: UO_2F_2, 40 mg/kg; uranyl nitrate hexahydrate, 128 mg/kg; UCl_4, 297 mg/kg.

6.2 LD$_{50}$ for 14 to 21 Days. Male and female rats given the three soluble compounds required much smaller amounts for an LD$_{50}$ when a longer time period was employed. The LD$_{50}$'s for uranyl nitrate hexahydrate and for UO_2F_2 were of the same order of magnitude and larger for males than for females; specifically, 2 to 2.5 mg/kg was the LD$_{50}$ for males, whereas the LD$_{50}$ for females was of the order of 1 mg. The comparable values for UCl_4 were larger; exactly how much larger is not known. The LD$_{50}$'s were possibly of the order of 5 mg/kg.

Male and female mice were studied in the same way as the rats. The LD$_{50}$ of uranyl nitrate hexahydrate for white mice was about 6 to 8 mg/kg; for C_3H mice, 20 to 25 mg/kg. This is a good illustration of the marked strain differences that sometimes exist.

6.3 Intravenous Mortality. Following intravenous administration in a limited preliminary study, the approximate lethal dose for rabbits was estimated to be of the order of 0.1 mg/kg; for guinea pigs, of the order of 0.3 mg/kg; for rats, of the order of 1 mg/kg; and for mice, of the order of 10 mg/kg.

7. STUDIES OF THE TOXICITY OF VARIOUS URANIUM COMPOUNDS WHEN FED TO EXPERIMENTAL ANIMALS

Ingestion studies were important because they helped to evaluate the toxic effects observed in the inhalation studies. All lung exposures are complicated by more or less ingestion of toxic materials. The program included (1) a good many 30-day feeding tests on rats, (2) a few chronic feeding tests on rats for periods of 2 years, (3) 30-day feeding tests on dogs, (4) 1-year feeding tests on dogs, and (5) a small feeding test on a few rabbits.

7.1 Thirty-day Feeding Tests, Rats. The various uranium compounds were divided into two groups on the basis of the mortalities in 30-day tests:

Nontoxic: UO_2, U_3O_8, UF_4.

Toxic: UO_3, uranyl acetate, UO_4, UCl_4, UO_2F_2, uranyl nitrate.

The lethal level to kill the average rat in a 30-day period was 1 to 4 per cent of UO_2F_2, UCl_4, UO_4, uranyl nitrate hexahydrate, UO_3, and uranyl acetate* in the diet. More than 20 per cent of UO_2, U_3O_8, and UF_4 must be required, since 20 per cent produced practically no mortality.

The compounds may also be grouped on the basis of the smallest percentage in the diets that will produce detectable growth depression.

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*Throughout this book the symbol Ac designates the acetate group and not the element actinium.

The compounds UO_2 and U_3O_8 are not included, since even 20 per cent of these compounds in the diets failed to produce growth depression.

Percentage in diet to produce minimum growth depression	Compound
0.1 – 0.25	UO_4, UO_2F_2
0.5 – 1	UCl_4, UO_3, $UO_2(NO_3)_2 \cdot 6H_2O$, UO_2Ac_2
20	UF_4

Pathological studies showed that consistent kidney damage followed the ingestion of the uranium compounds.

Careful studies of the food intake showed that partial inanition followed promptly when rats were placed on diets containing toxic amounts of the various uranium compounds. Apparently each rat ate his habitual amount of food on the first day that the uranium compound was placed in the diet. Thereafter the dietary intake decreased, and a fall in body weight soon followed. Later, in surviving rats the reverse relationship held; food intake increased rapidly, followed after a day or two by body-weight increase.

7.2 One-year Feeding Studies, Rats. Four of the compounds were studied over a longer feeding period: UO_2F_2 in amounts up to 0.5 per cent of the diet, uranyl nitrate hexahydrate in amounts up to 2 per cent of the diet, and UF_4 and UO_2 in amounts up to 20 per cent of the diet. During the first few months there were growth retardations that increased with increasing amounts of the toxic materials. By the sixth month the growth curves tended to flatten, and little further growth occurred. Mortalities for all groups of rats receiving 0.25 per cent UO_2F_2 or less, 2 per cent uranyl nitrate hexahydrate or less, and 20 per cent UF_4 and UO_2 or less were the same as those of the control groups, namely, 10 to 20 per cent by the twelfth month.

A breeding experiment was carried out in which pairs of rats fed 2 per cent uranyl nitrate hexahydrate were compared with pairs of control rats. From the start the uranyl nitrate-fed rats had fewer litters and fewer young per litter. After 7 months the experimental rats were again placed on the control diet and in the succeeding 5 months regained most of the lost body weight. No such sudden increase in the numbers of litters or in the number of young accompanied the growth impetus.

7.3 Two-year Feeding Test, Rats. The tendencies described, which were established by the sixth month and were maintained during the balance of the first year, were continued with only minor fluctuations during the second year on the same diets. No interference with life span was observed in the rats fed up to 0.25 per cent UO_2F_2, 2 per cent uranyl nitrate hexahydrate, or 20 per cent UF_4 or UO_2. Sixty to seventy per cent of the control rats had died by the twenty-fourth month; 34 to 82 per cent of the experimental rats had died in the same period.

7.4 Thirty-day Feeding Tests, Dogs. A series of feeding studies were conducted in which a single dog was fed a certain amount of each uranium compound for a period of 30 days. The criterion of toxicity was survival for the feeding period. On the basis of these studies, the compounds are divided into the following three groups:

Degree of toxicity	Compound	Amount and comment
Highly toxic	UO_2F_2, UCl_4, UO_4	0.01 g/kg/day tolerated; 2 to 20 times this amount is lethal
Moderately toxic	$UO_2(NO_3)_2.6H_2O$, UO_3, $Na_2U_2O_7$, $(NH_4)_2U_2O_7$, High-grade ore	0.1 to 0.5 g/kg/day tolerated; 5 to 10 times this amount is lethal
Slightly toxic	UF_4, U_3O_8, UO_3	5 to 20 g/kg/day tolerated

Pathological studies showed the typical kidney lesions of acute uranium poisoning. In addition there were signs of injury, and in severe poisoning there were hemorrhages in the gastrointestinal tract. There were various degrees of liver-cell changes: abnormal fat, necrosis, and degeneration.

Comparing the results of 30-day tests on dogs with those on rats, it was concluded that 10 to 50 times as much of the various materials that produced injury in the dog would be required to produce injury in the rat.

7.5 One-year Feeding Test, Dogs. Year-long studies were conducted on a few dogs that were fed up to 0.01 g/kg/day of UCl_4 and up to 0.2 g/kg/day of uranyl nitrate hexahydrate. In general, soluble uranium compounds apparently produced obvious evidence of toxic effects if given in excess of 0.02 g/kg/day. Studies will be reported later in which UO_2 was fed in amounts up to 10 g/kg/day and UF_4 was fed in amounts up to 5 g/kg/day.

One of the most interesting aspects of the chronic feeding studies in dogs is the question of whether a cyclic pattern of injury develops during the chronic ingestion of these compounds. There was a repeated tendency for a sharp increase in positive signs of uranium poisoning to be seen immediately after diets were instituted containing these compounds. Then, after a normal period of many weeks (perhaps 15 or 20), another major symptom of poisoning, e.g., high blood NPN values, could be observed.

Comparing the data obtained in the 1-year studies on dogs and on rats, it appears that toxic signs are produced under those conditions in a dog by about one twenty-fifth as much UO_2F_2 as gives detectable changes in rats; by about one-twentieth as much uranyl nitrate hexahydrate; and by about one-fourth as much UF_4.

7.6 Thirty-day Feeding Test, Rabbits. A few rabbits were fed diets containing various amounts of uranyl nitrate. All the rabbits fed 0.5 per cent uranyl nitrate hexahydrate died; more than half those fed 0.1 per cent died; but none fed 0.02 per cent died in the 30-day period. The data obtained from these experiments may be compared with data from dog- and rat-feeding tests. The lethal level to kill half of the group within 30 days is about 0.04 g/kg/day in rabbits, 0.1 in dogs, and 4 in rats. There is roughly a fourfold increase between each species. The amounts to produce a just-detectable weight depression are about 0.01 g/kg/day in rabbits, 0.1 to 0.2 in dogs, and 0.5 in rats. Expressed in this way, there is a factor of 5 to 20 between the susceptibilities of the various species.

8. THE TOXICOLOGY OF COMPOUNDS OF URANIUM FOLLOWING APPLICATION TO THE SKIN

The studies on dermal toxicology may be conveniently considered under four heads: (1) the toxicity following a single dose applied to the skin, (2) the toxicity following the application of repeated doses to the skin, (3) the toxicological aspects of protective coatings and of detergents, and (4) laundry procedures for the removal of uranium compounds from cloth.

8.1 Single Dose. A great deal of work was done using solutions of uranyl nitrate in ether. A marked variation in species susceptibility was discovered. The LD_{50}'s reflected these variations: that for rabbits was 0.06, that for rats was 0.5, that for guinea pigs was 2.1, and that for mice was about 7.6 g of uranium per kilogram of body weight. No relation was discovered between body weight and mortality in a given species.

Some work using UCl_4 in guinea pigs showed the LD_{50} to be of the order of 4 g of uranium per kilogram of body weight.

At least three of the compounds were moderately irritating to the skin locally. These were uranyl nitrate, UCl_4, and UCl_5. All these compounds produce acids on hydrolysis.

On the basis of pilot studies, the various uranium compounds were grouped roughly into three groups as regards their toxicity when applied to the skin:

1. Highly toxic: uranyl nitrate, UO_2F_2, UCl_4, UCl_5.
2. Moderately toxic: UO_3, $Na_2U_2O_7$, and $(NH_4)_2U_2O_7$.
3. Nontoxic: UF_4, UO_2, UO_4, U_3O_8.

The symptoms in animals that were poisoned were those usually observed in uranium poisoning: the blood NPN rose, protein was found in the urine, histopathological changes were discovered in the kidneys, and losses in body weight were noted.

8.2 Chronic Administration. Considerable investigation was carried out on repeated administration of small doses in an effort to discover something of the cumulative nature of the poisoning via this route of administration. When a large dose such as 1.3 g/kg was given three times per week to guinea pigs, a 5 per cent mortality was observed in four doses, with the cumulative dose being 5.4 g/kg. This may be compared with the approximate LD_{50} of 2.1 g/kg when a small dose, e.g., 0.1 g/kg was given six times per week; a 5 per cent mortality was observed after about 13 doses. It was clearly possible to produce severe poisoning by repeated small doses.

8.3 Protective Coatings. Several studies were devoted to finding the effect on the course of poisoning of (1) the use of some of the creams and protective coatings currently employed by industrial personnel and (2) some other common oily materials. Various detergents were also used as another aspect of the same problem. No significant changes in mortality were found.

8.4 Laundry. The problem of removing uranium from clothing had an obvious importance. It was found that soaps would not remove uranium efficiently. However, various complexers were quite successful. Among these may be mentioned bicarbonate, carbonate, oxalate, and citrate. A 2 per cent solution of bicarbonate was judged to be the best for the purpose.

9. THE TOXICOLOGY OF COMPOUNDS OF URANIUM FOLLOWING APPLICATION TO THE EYE

Among the hazards of industrial operations, one most carefully guarded against is the accidental entrance of chemicals into the eye. Such studies were undertaken to indicate which of the uranium compounds was to be feared because of the local irritant action. In the course of these studies it was found that several of the compounds were capable of producing acute uranium poisoning when introduced into the eyes. Some were found, especially UCl_5 and UCl_4, that are extremely dangerous locally and should never be handled without adequate tight-fitting goggles.

For the most part, the studies were made by placing single doses of the compounds in the conjunctival sac of rabbits. Limited studies were done on guinea pigs and rats. The compounds were introduced in various forms as dry powders, aqueous solution, and suspensions in lanolin or in water.

Observations were made on the conjunctivas to discover the presence of inflammation, edema, exudation, and ulceration. The corneas were also observed for the four following changes: cloudiness, corrosion, ulceration, and vascularization.

On the basis of the damage produced to the eye, the compounds tested may be provisionally divided into the following three groups:

1. Severe damage was produced by UCl_5, UCl_4, $UOCl_2$, UO_2F_2, uranyl nitrate, and $Na_2U_2O_7$.
2. Moderate damage was produced by UF_4 and $(NH_4)_2U_2O_7$.
3. Mild damage was produced by UO_3, talc, UO_2, UO_4, and U_3O_8.

In a number of cases, poisoning severe enough to be evident and even produce mortality was produced by the following compounds: uranyl nitrate, UO_2F_2, UCl_4, UCl_5, UO_3, $Na_2U_2O_7$, and $(NH_4)_2U_2O_7$. Repeated doses of UF_4 in the eyes of rabbits produced mortality.

10. TOXICITY FOLLOWING INHALATION

10.1 Introduction. What has resulted in undoubtedly the largest inhalation project ever undertaken on heavy-metal poisoning was begun under war-emergency conditions to fulfill the vital need of protecting workers in a newly arisen uranium industry. The inhalation project was started with the benefit of little more than cursory prior experimentation in the field but was completed within the assigned time with all major objectives achieved and with numerous contributions to the field of uranium toxicity to its credit.

10.2 Materials and Methods. A number of designs of exposure chambers were tested; a number of devices to perform the more difficult task of producing a high-density uranium dust were constructed. Out of the experiences, satisfactory designs of both chambers and dust feeds have been evolved. Methods of sampling and of testing dust concentrations have been standardized. Improved methods of sampling and measurement of dust-particle sizes also have been made. These have comprised the redesign of the British Cascade Impactor for dust sampling and the application of the high-refractive-index-coating technique for the extension of measurements to smaller particle sizes with concomitant increase in definition. Of basic importance to the understanding of problems of inhalation of insoluble uranium dusts, and probably of insoluble dust in general, was the finding that particles of uranium dust, UO_2, smaller than 1μ were far more toxic than particles of larger sizes. Remarkable was the demonstration that the smaller particles were transferred out of the lungs at a slower rate than were the larger particles, although the smaller particles were responsible for the greater deposition of uranium in the kidneys, the primary site of uranium damage. Undoubtedly different modes of transfer are involved.

A number of toxicologic procedures that should find specific as well as general usefulness in large-scale studies have been employed. Among these are specific biochemical tests of renal function, used prior to experimentation in order to cull animals unsuited for tests involving renal damage. A conditioning period prior to exposure has been considered another essential in inhalation-exposure work. The techniques of serial histological examination of animals that are repeatedly exposed and of renal biopsies to trace the course of toxicity in a single animal have been highly satisfactory procedures.

10.3 Results of Short-term Exposures. A total of forty-six 30-day tests have been made in which various groups of animals have been exposed to concentrations of 12 different dusts (from 20 to 0.05 mg/cu m). In addition two 90-day control studies have been carried out in which no special handling of the animals was attempted. In two additional 30-day studies of control animals all the details of exposure in a chamber were faithfully adhered to; the sole difference from the experimental studies was that no toxic dust was added to the atmosphere.

(a) Control Studies. The animals observed in the control tests are important for several reasons, the principal one being that these studies supplied the base line of pathological changes, blood and urine chemistry values, and mortalities for the interpretation of comparable observations on experimental animals.

A careful summary of detailed pathological studies of the tissues of dogs, rabbits, guinea pigs, and rats has shown a widespread occurrence of various mild abnormalities. A large percentage of the dogs suffered from a mild chronic interstitial nephritis. In rabbits the appearances were similar, although more advanced, and were described as moderate chronic interstitial nephritis. In rats and guinea pigs these changes were only occasionally seen. Parasites in dogs and rabbits were frequently observed; 5 of 20 dogs and 37 of 70 rabbits showed some form of parasitism. Mild pulmonary pathologies were frequently observed in guinea pigs.

From chemical determinations several interesting observations were recorded. Protein was found to be present in 15 out of 102 analyses on dog urines, whereas none of 214 tests on rabbits were positive. Determinations of urinary sugar were positive in 8 out of 101 dogs, whereas 4 of 214 were positive in rabbits. Catalase determinations on rabbit urines gave values exceeding 50 cu mm of O_2 in 8 out of 158 cases; this is the catalase value above which values were considered abnormal. Urinary phosphatase values were positive in 15 out of 183 cases.

One of the useful observations was the spread in values obtained in hematological examinations among the several species in these studies.

The over-all mortalities were 16 of 300 animals, or 5 per cent. Six of the deaths were attributed to natural causes such as old age and pneumonia. Ten animals were accidentally killed in handling.

(b) UF_6. The compound UF_6 is a gas at ordinary temperatures and pressures. In contact with moist air, it hydrolyzes instantly with the formation of HF and fume particles of UO_2F_2 (particle size less than 0.5μ).

A series of flood-concentration studies were first undertaken in which 1,000 to 2,300 mg/cu m was employed. Later 30-day studies were conducted at concentrations of 20, 3, 0.3, and 0.08 mg/cu m (equivalent to 13.3, 2.0, 0.2, and 0.05 mg U/cu m).

The highest concentration was highly lethal. The mortalities ranged from 100 per cent in mice to 40 per cent in dogs. The species in order of susceptibility are as follows: mice and rabbits are more susceptible than rats, which are more susceptible than guinea pigs and dogs.

Many toxic effects were observed. Blood NPN elevations were found in exposures to the two higher concentrations. Severe tubular damage was observed at the highest concentration at which lung pathology was also found, perhaps attributable to the HF. Pathological changes were described as mild at 0.3 mg/cu m and were not observed at 0.08 mg/cu m.

The flood concentration gave the usual toxic uranium changes and in addition produced irritation of the eyes, nose, and mucous membranes.

(c) UO_2F_2. Several species of animal were placed in exposure chambers and studied for periods of 30 days in atmospheres containing 12, 2.5, 0.6, 0.2 mg UO_2F_2/cu m (equivalent to 9.2, 1.9, 0.46, and 0.15 mg U/cu m).

Uranyl fluoride at 12 mg/cu m killed over 50 per cent of the animals. At 2.5 mg/cu m only rabbits and mice were killed. A concentration of 0.6 was lethal to mice only, and there was no mortality at 0.2 mg/cu m. Mice were the most susceptible species, then, in order of decreasing susceptibility, rabbits, dogs, cats, guinea pigs, and rats.

Weight loss was observed at the highest level, but not much change was found at 2.5 mg/cu m or below. Elevations in NPN of the blood were found as were proteinuria, catalasuria, and increased amino acid nitrogen excretion.

In a series of important studies of kidney clearances, marked changes were found at the highest level. The chloride clearance was increased, and diodrast and inulin clearances were decreased. These results were interpreted as evidence of profound interference with the normal functions of the proximal convoluted tubule and of filtration. Pathological changes that were very severe in animals exposed to 12 mg/cu m were only slight in dogs and rabbits at 0.6 mg/cu m and negligible in animals exposed at 0.2 mg/cu m.

Uranyl fluoride is considered one of the most toxic uranium compounds.

(d) Uranyl Nitrate. A number of special studies were made in connection with the exposures to $UO_2(NO_3)_2.6H_2O$ dusts. Studies were carried on at concentrations of 20, 4.5, 1.9, 0.5, 0.3 mg/cu m (equivalent to 10.4, 2.11, 0.9, 0.25, 0.14 mg U/cu m). Mortality was observed most frequently in rabbits and cats; dogs died only at 20 mg/cu m, as did guinea pigs and rats. A few mice died at the lower dust concentrations, presumably not as a result of dust exposure. Body-weight loss occurred at the highest concentration, none at 0.3 mg/cu m. There was some question as to whether changes in the hemoglobin picture followed the exposure at 20 mg/cu m.

Pathological changes were severe in kidneys of dogs at 20 mg/cu m; also pulmonary injuries were present. Serial studies of rats were made at 4.5 mg/cu m and these showed sequence of injury less than that of the rabbits. Slight to moderate changes were observed at 1.9 mg/cu m; and at 0.5 mg/cu m severe changes were seen in the rabbits, but only slight changes in the rats. At 0.3 mg/cu m only slight changes were observed.

Chemical determinations showed the occurrence of elevated blood NPN and depressed blood CO_2 in dogs at 20 mg/cu m. The glucose tolerance curve in these animals was prolonged. Proteinuria and glycosuria were found. Rats exposed to the same atmospheres exhibited no acidosis at 14 days but were acidotic at 30 days and showed elevated blood NPN values. Surprisingly enough, administration of a bicarbonate-containing diet to these rats served to increase the mortality. Studies of the carbohydrate metabolism in rats revealed elevated blood glucose in the tolerance test, and, whereas no change in liver glycogen was found, muscular glycogen values were low. Some chemical changes were seen at 4.5 mg/cu m and less at lower concentrations. In a study of animals exposed to 0.5 mg/cu m, urinary catalase and phosphatase, proteinuria, blood NPN, and kidney pathology were shown to be equally well correlated in the course of acute uranium poisoning.

Studies on certain dogs and rats at 0.3 mg/cu m showed that there was no change in the prothrombin level of the blood fibrinogen and a decrease in some animals in the bromsulfalein excretion.

Clearance tests were carried out on dogs at two levels. The inulin clearance fell at 1.9 mg/cu m, and later the diodrast level fell, but no change occurred in the chloride clearance. At 0.3 mg/cu m no change was found in the inulin clearance, no changes were seen in the diodrast clearance, and the chloride clearances were increased.

(e) $\underline{UCl_4}$. The compound UCl_4 as a dust is hygroscopic so that the dust rapidly becomes a liquid droplet in which hydrolysis and oxidation partly convert the U_4 to U_6 and HCl. Animals exposed to this material in high concentrations have matted green fur and green-black deposits on the teeth.

A series of experiments was carried out at 18, 3.3, 1.8, 0.3, 0.2, 0.1 mg/cu m (equivalent to 11, 2.1, 1.1, 0.2, 0.1, 0.06 mg U/cu m). Mortality was highest in the rabbits and mice, next in the rats, and least in the guinea pigs. No mortalities were observed in dust concentrations of 1.8 mg/cu m or less. Some weight loss was observed in animals exposed to concentrations of 1.8 mg/cu m or more. The blood NPN increased in the experiments at the high dust concentrations. Elevation in urinary protein levels was also observed, though none was found in exposures to 0.3 mg/cu m or less. Urinary amino acid nitrogen increased at 18 mg/cu m but not at 0.2 mg/cu m.

Pathological changes were found, severe at the highest concentrations, extremely mild at the lowest. The renal pathology was the typical tubular necrosis, which was severe at 18 mg/cu m, mild to moderate at 1.8 mg/cu m, and extremely mild at 0.3 mg/cu m and less. An unusual pathological finding was the pulmonary hemorrhage and edema seen in rabbits exposed to 18 mg/cu m and to a lesser degree at 3 mg/cu m.

(f) $\underline{UF_4}$. Thirty-day exposure studies have been made of several species of animals that breathed air containing UF_4 dust in concentrations of 24, 5, 4, 0.7, and 0.5 mg/cu m (equivalent to 18, 3.8, 3, 0.6, and 0.38 mg U/cu m).

Mortality was observed at only the highest level, although signs of pathological changes were seen down to but not including the exposure at 0.5. Interestingly enough, even at this level, rabbits exhibited some evidence of catalasuria. Marked elevations of blood NPN were found at the highest concentration. Elevations of urinary amino acid nitrogen were found in rabbits exposed to 3 mg/cu m.

The compound UF_4 is considered to be one of the least toxic of those tested.

(g) $\underline{UO_2}$. This brown oxide is a typical insoluble uranium compound. Dogs, rabbits, guinea pigs, rats, and mice were exposed to atmospheres with 22, 10.4, 9.3, 2.0, 1.3 mg/cu m (equivalent to 19, 9.2, 8.2, 1.8, and 1.1 mg U/cu m). Mortality was marked only in the highest concentration, e.g., 60 per cent of the rabbits died, but only a few deaths were recorded at lower concentrations. Body weight decreased at 22 mg/cu m; otherwise there were no changes. The blood NPN of rabbits increased in exposures at the highest levels. Pathological changes were severe in rabbits at 22 mg/cu m. No pathology was observed save renal changes in six dogs exposed at 9.3 mg/cu m.

(h) High-grade Ore. Uranium ores contain other toxic materials, notably lead (6 per cent PbO), nickel (1 per cent Ni), and cobalt (0.4 per cent Co). Three dust-exposure studies were carried on at 36, 1.8, and 0.4 mg/cu m (equivalent to 20, 2.9, and 0.84 mg U/cu m). The degree to which lead may have entered into the picture is emphasized by considering that, at a dust concentration of 36 mg/cu m, 2 mg of lead per cubic meter was present, and that at a dust concentration of 4.8 mg/cu m there was 0.29 mg of lead per cubic meter in the air, or twice the allowable concentration. Determinations of both lead and uranium were conducted on air samples. The lead-uranium ratio did not vary appreciably, indicating that no separation occurred in the air dispersal.

Mortality was negligible save in some rabbits that unfortunately were suffering from coccidiosis. Only questionable changes in body weight occurred. Some elevation of blood NPN was observed in rabbits at 36 mg/cu m, but only questionable changes at 4.8 mg/cu m. Pathological changes in both lung and kidney were moderately severe at the highest dust concentration and decreased with decreasing concentrations, so that at 1.4 mg/cu m a slight kidney injury in three of five dogs was the only pathological finding.

Uranium and lead analyses were made of many tissues. Small quantities of uranium were found in all the tissues except liver; in contrast, lead was found in only lung and bone. In the latter tissue, values twice the normal ones were found.

(i) UO_3. The solubility of this compound is considerably higher in plasma than in water. Consequently it was somewhat more toxic than might have been expected. A single study was carried out on dogs, cats, rabbits, guinea pigs, rats, and mice at 20 mg/cu m. Under these conditions UO_3 was highly toxic to cats and rabbits, but less so in dogs, guinea pigs, rats, and mice. Body-weight losses were found; blood NPN increased. Pathology studies showed no changes in brain tissue, mild to severe kidney injury, lung damage in some rabbits, fatty liver sometimes in rabbits and rats, and questionable involvement of bone marrow. A group of five rabbits was studied at the 10th, 19th, and 28th days. Decreased excretion of creatinine, diodrast, phenol red, and urea was found regularly, with the exception of two determinations on one of the rabbits. The diodrast values were very low in one of the three rabbits, and chloride clearances were uniformly increased.

(j) $\underline{UO_4}$. A single study of UO_4 dust was carried out in which a dog, cats, rabbits, guinea pigs, rats, and mice were exposed to atmospheres containing 19.5 mg/cu m (equivalent to 13 mg U/cu m) for 23 days. Surprisingly enough, UO_4, despite its low water solubility, was lethal for all species. The single dog was sacrificed but not because of advanced uranium poisoning. The mortalities in the other species were as follows: cats, 100 per cent; rabbits, 80 per cent; guinea pigs, 40 per cent; old mice, 63 per cent; young mice, 23 per cent; and rats, 10 per cent.

Customary signs of uranium poisoning were found. There were weight losses, blood NPN elevations, and renal pathological changes of mild to moderate degree.

Although it is highly insoluble in water, UO_4 is fairly soluble in serum and is highly toxic when inhaled as a dust.

(k) $\underline{Na_2U_2O_7}$. In a single study of the sodium salt, groups of dogs, cats, rabbits, rats, guinea pigs, and mice were exposed to 30 mg/cu m (equivalent to 15 mg U/cu m). Following this exposure study a number of animals were studied in more detail for a succeeding 13 weeks.

Rabbits exhibited the highest mortality and guinea pigs and mice next, whereas dogs, cats, and rats did not die. The body weight fell in most animals. Renal injury followed the usual pattern of severe necrosis and regeneration. The changes were more severe in dogs and rabbits than in rats. Studies of the blood chemistry showed decreased CO_2, i.e., acidosis, and decreased lactate. Urinary chemical studies showed initial increases and, later, decreases in creatine, protein, lactate, and urinary volume. Urinary ammonia was observed to increase and to remain abnormally high. Creatinine remained normal.

In the postexposure period, clearance studies showed fairly normal values for diodrast and inulin clearances. Chloride clearances in contrast were increased to about double normal values.

(l) $\underline{(NH_4)_2U_2O_7}$. A single study was carried out on the toxicity of the ammonium salt as a dust. Groups of rabbits, rats, guinea pigs, and mice were exposed to atmospheres containing 20 mg/cu m (equivalent to 12 mg U/cu m). The rabbits suffered the highest mortality, mice the next highest. Although some guinea pigs died, the data were not clear cut because an infection was present in the colony at the time. Rats were most resistant; none died.

The expected signs of uranium toxicity were observed. The body weights decreased in most animals. Severe pathological changes were seen in the rabbits, but rats studied serially showed severe injury followed by recovery.

Two signs of toxicity not usually observed were attributed in part to the nature of the salt and perhaps to its ammonium content. First, nasal irritation was a common finding. Second, hematological changes were observed, increased amounts of neutrophils and lymphocytes were seen, and, in rats, there was a drop in the red-blood-cell count.

(m) U_3O_8. A single study of the toxicity of U_3O_8 when inhaled as a dust was carried out on rabbits, guinea pigs, rats, and mice exposed for 26 days to atmospheres containing 17 mg/cu m (equivalent to 15 mg U/cu m). The exposures in rabbits were complicated by the presence of an infection so that the eight deaths recorded among the 45 animals were presumed not to be a reflection of the toxicity of the compound. There were moderate renal tubular changes. Under the conditions studied, U_3O_8 is not very toxic.

10.4 Respiratory Protective Devices. One question that immediately was raised in regard to the health of workmen in dusty atmospheres was what protection would be afforded by respirators and canisters. To answer this question a series of studies was carried out in one of the exposure chambers. Fifteen respirators and seven canisters were tested against one or more concentrations of nine dusts. Comfort ratings were made as well as statements of acceptability on the basis of penetration tests. The tests were usually run in triplicate on respirators, whereas only one canister was tested. Testing procedures as worked out by the U. S. Bureau of Mines were employed. The specific ratings are given in Table 10.119.

10.5 Discussion. Chamber Construction and Dust-feed Problems. Most of the exposure chambers were cubes of the order of 6 ft on an edge. Various modifications and variations were undertaken, the principal one being the construction of units designed to allow exposures limited to the heads of the experimental animals. Although some of these have been in operation for a year, it is widely believed to be an unnecessary and time-consuming refinement. In the use of certain corrosive materials, special linings of the chambers were provided, e.g., copper or lead, but for most of the chambers corrosion-resisting paints were found to be sufficient.

The dust concentrations were individual problems. A number of duct feeds were tested. Satisfactory results were obtained only when the feed emptied directly into the interior, any duct work between the feed and the chamber being omitted. Thorough mixing of the dusty atmosphere was maintained by keeping a turbulent air flow. The animals were kept in individual cages so as to rule out factors of crowding. The filter-paper dust sampler modified from Fairhall's original model proved entirely satisfactory in most cases.

Characteristics of the Dust. Many problems arose in connection with particle size of the dust. Gratifying success in measuring particle size resulted from the use of devices such as the Cascade Impactor, the electron microscope, and the coating of dust particles with a medium (selenium) of high refractive index. Particles less than 1 μ

are known to be more toxic than larger particles. The density of the uranium dusts was an added difficulty. Wide variations in the solubility of uranium dusts introduced wide variations in toxic effects.

The Acute Toxic Response. A number of uranium dusts were studied at several air concentrations. The results served to define the dust concentrations that are certainly toxic, dust concentrations where only mild transitory injuries were observed, and dust concentrations where there were no detectable changes. These concentration-response relations are quite different for soluble and for insoluble dusts, as is indicated in Table 3.

Table 3 — Acute Toxic Response

Approx. dust concentration	UF_6, UO_2F_2, UCl_4, $UO_2(NO_3)_2.6H_2O$		UF_4, high-grade ore, UO_2	
	Mortality	Kidney damage	Mortality	Kidney damage
20	High	Severe	Occasionally	Moderately
2.0	In some species	Severe to moderate	Rarely	Mild or none
0.2	Occasionally	Slight	None	None
0.05	None	None	None	None

Criteria. During the course of the studies many techniques were applied to poisoned animals to determine what effects were measurable. Some of these tests were ultimately shown to be almost completely uninformative. For example, the careful hematological studies in almost no instances showed aberrations attributable to uranium poisoning. On the other hand, certain examinations, e.g., blood NPN and urea nitrogen; urinary protein and other chemical tests; pathological examinations, particularly of the kidney; and renal function tests, especially of renal clearance, were widely used and were extremely reliable criteria of uranium poisoning. Of all these tests, it was generally agreed that the most sensitive indicators of early damage were the biochemical tests, catalasuria, proteinuria, and the ratio of urinary amino acid nitrogen to creatinine; histological changes were only slightly less sensitive.

Characteristics. Among the characteristic effects that form the pattern of acute uranium poisoning none stands out more clearly than the lag period between the initial exposure and the height of the poisoning. It will be seen from the detailed studies that several days to a week regularly elapsed between the beginning of exposure and the development of a toxic effect. Another remarkable characteristic was the prompt regeneration and healing of damaged kidney structures in the surviving animals. By many tests, animals that initially had given evidence of severe intoxication appeared normal at the end of a 30-day exposure. Thus, in Fig. 1, the course of two criteria, body weight and blood NPN, are diagrammed in the time sequence of changes following a moderate-sized toxic dose.

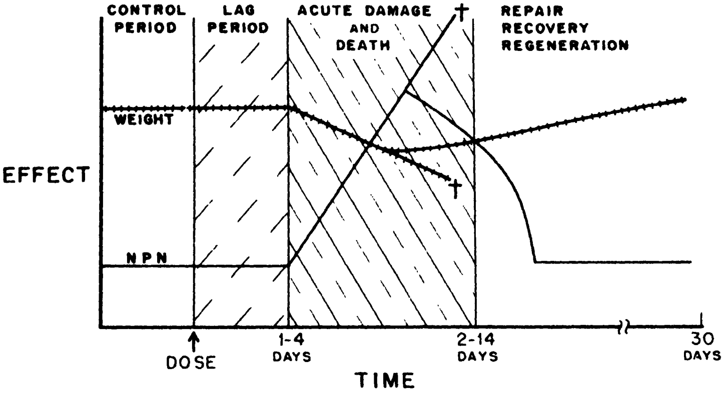


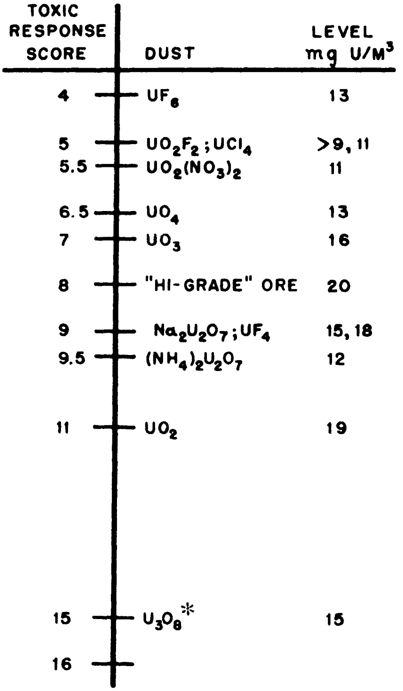
Fig. 1 — Time course of acute poisoning.

Species Susceptibility. It is somewhat perilous to indicate species susceptibility to uranium compounds in general, because so much variability was observed from compound to compound. However, in general the most susceptible species were the rabbit and cat. The dog and mouse were of mid-susceptibility. The least susceptible were the guinea pig and the rat.

Relative Toxicity. Based on four criteria, namely, mortality, weight loss, biochemical changes, and histological changes, numerical scores were given to results of exposures of dogs, rats, and rabbits to many compounds at many concentrations. The scores are arranged so that the most toxic score is the smallest number. The least toxic score is the largest number. This combination of criteria permitted various compounds to be rated as to their toxicity on the scale shown in Fig. 2.

Role of Ingested Uranium. In the best comparison that has been found possible, various uranium compounds appear to be much more toxic by lung than by mouth. In fact, different compounds ranged from 65 to 10,000 times as toxic when inhaled in the air as when swallowed. Despite this higher toxicity by lung exposure it has been estimated that ingested material may contribute up to 15 per cent of the toxic effect in lung exposed at levels approximating 20 mg/cu m, especially in rats and mice.

Retention. There is little doubt that one of the most significant numbers in connection with lung exposure is the percentage of the dust, taken in by breathing, that is retained in the body. Wide variations were predicted. In most cases no estimations have been made.



* THE POSITION OF U_3O_8 IS FIXED WITH LESS CERTAINTY THAN THE OTHERS BECAUSE OF LESS DETAILED STUDY.

Fig. 2—Relative toxicity by inhalation of twelve uranium dusts. Greatest toxicity, lowest possible score, 4; lowest toxicity, highest possible score, 16.

To illustrate the order of magnitude that may occur, it has been calculated that in rabbits 20 to 50 per cent of uranyl nitrate hexahydrate dust at 0.5 mg/cu m was retained.

11. DISTRIBUTION AND EXCRETION OF URANIUM

When uranium compounds are administered intravenously, hexavalent and tetravalent forms are distributed through the body, stored, and excreted in characteristic but different patterns. In some respects, especially in bone storage, distributions are closely comparable for U_6 and U_4. In other respects, especially in urinary excretion rates, the patterns are sharply dissimilar. Figure 3 shows the fate of injected uranium, U_6 and U_4, at two time periods, 45 min and 40 days, after the administration of a single dose.

11.1 Blood. The blood contains about 10 per cent of the dose of U_6 after an hour, whereas 50 per cent of the U_4 is held in the blood at that time. This difference is in line with the comparatively strong protein-binding property of U_4, whereas U_6 complexes with bicarbonate to a greater extent and is therefore more diffusible. Both forms are practically absent from the blood by 40 days.

11.2 Bone. For both U_6 and U_4, bone deposition is prompt, important, and permanent. Fifteen to 30 per cent of the injected dose is immediately deposited in the bone, and scarcely any is lost from this tissue in the succeeding 40 days.

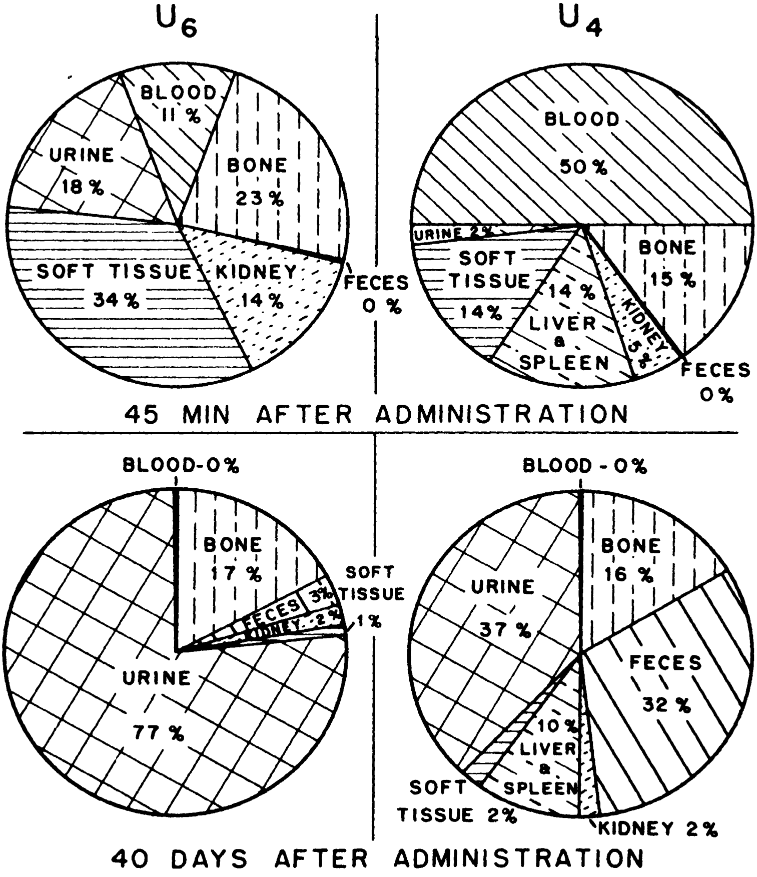


Fig. 3—Distribution and excretion of uranium.

11.3 Urine. The tendencies for U_4 to complex with protein and for U_6 to complex with bicarbonate are reflected in the urinary excretion rates. The U_6 is rapidly excreted in urine, upward of 20 per cent in an hour, whereas one-tenth of this amount of U_4 has been excreted in the same time. Furthermore some 15 per cent of the U_6 is found in the kidney after about an hour as compared to one-third this amount of the U_4. About one-third of the dose of either form is present in the soft tissues after 1 hr; however, the fate of this part is quite different for U_6 and for U_4. The U_6 present in the kidney and in the other soft tissues and in the blood at 1 hr is excreted via the urine in the ensuing days. Most of the excretion occurs in the first week, and smaller amounts are excreted in the succeeding weeks, so that, at 40 days, three-quarters of the U_6 has been excreted in the urine. In the case of U_4, much larger amounts than are present in the liver and spleen at 1 hr later appear in these organs. As much as 50 per cent of the dose may be found in the liver from 3 to 8 hr after administration. This is in large measure excreted so that only about 10 per cent is present after 40 days. Furthermore the U_4 is excreted almost equally in the feces and in the urine, so that at 40 days only a little over one-third of the U_4 administered has been excreted in the urine.

With both U_6 and U_4 it should be emphasized that 75 to 80 per cent of the dose has disappeared from the body in 40 days and that most of the retained uranium is in the bone.

12. TOLERANCE TO URANIUM POISONING

One of the significantly important aspects of uranium toxicology is the development of resistance to some of the poisonous effects. This phenomenon, frequently described as "tolerance," may be defined in at least two ways: (1) a lessened response to a repeated dose of the same size and (2) an increase in dose to reproduce an initial response.

12.1 Natural Tolerance. Another way of expressing the variation in species susceptibilities is by natural tolerance. Several examples may be cited:

(a) Intraperitoneal Injection. Rats were more susceptible than were white mice, which in turn were more susceptible than were C_3H mice. Numerically, LD_{50}'s were as follows: rats, 1 to 2.5 mg/kg; white mice, 6 to 8 mg/kg; C_3H mice, 25 mg/kg.

(b) Intravenous Injection. Rabbits were more susceptible than were guinea pigs, which in turn were more susceptible than were rats, which were more so than white mice. Numerically the lethal doses were approximately as follows: rabbits, 0.1 mg/kg; guinea pigs, 0.3 mg/kg; rats, 1 mg/kg; white mice, 10 mg/kg.

(c) Percutaneous Administration. Following the administration of various compounds on the skin, rabbits were found to be more susceptible than rats, which were more susceptible than guinea pigs. White mice were the most resistant of the species tested. Numerically, expressed as amounts of the nitrate applied to the skin, the LD_{50}'s were of the following magnitudes: rabbits, 0.1 g/kg; rats, 1 g/kg; guinea pigs, 4 g/kg; white mice, about 16 g/kg.

(d) Ingestion. Rabbits were more susceptible than dogs, which were more susceptible than rats. There was roughly a tenfold progression in susceptibility between each of the three species.

One of the major factors in natural tolerance has been shown to be the age of the animal. In general, the animal becomes much more susceptible to acute uranium poisoning as it becomes older.

12.2 Acquired Tolerance. By the method of repeated doses given intraperitoneally to rats, it has been shown that a marked degree of resistance can be built up over a period of several weeks. Thus a test dose of 5 mg/kg in untreated rats caused high mortalities, whereas in rats that had previously received frequent doses of 0.3 mg/kg little or no mortality followed the test dose. Haven demonstrated that the 0.3 mg/kg dose was a critical one. In studies thus far limited to adult female rats, 0.2 mg/kg gave only partial protection, and at 0.1 mg/kg there was no evidence of the establishment of tolerance at all.

In animals that were treated repeatedly with small doses of uranium compounds, a partial inanition and loss of weight was usually observed. Since the test dose was given as 5 mg/kg, the smaller treated rat received a smaller dose. A careful experiment, in which the control rats were placed on a restricted dietary intake so that their weights coincided from day to day with those of the treated rats, revealed the fact that there was no relationship between the inanition effect and tolerance. The control rats suffered almost 100 per cent mortality, but the treated rats suffered little or no mortality after the test dose.

Tolerance is not developed immediately. In fact, there seems to be an optimum period between the repeated doses for the development of tolerance. Ten days seems to be a better time interval between repeated doses than 20 days. It has also been found that tolerance was lost when the repeated exposures were discontinued; however, the loss was not immediate, and some resistance was maintained for considerable periods.

12.3 Mechanism of Tolerance. It seemed that one of the reasons for the lessened poisonous effect in tolerant rats was that less uranium was retained in the kidneys following the test dose.

An amazing pattern of citrate excretion in rats given uranium has been discovered. A few days after the administration of uranium, citrate excretion rose to a sharp peak of several times the normal excretion rate. By the sixth day the citrate excretion had returned to normal. By the tenth day a second period of increased excretion began; the urinary output frequently rose to levels six or more times normal and was maintained with some fluctuation for periods of approximately 10 days. The significance of these peaks is unknown at the present time. However, it appeared that the administration of the repeated doses must come at the time of the second peak in citrate excretion to be most useful in building up tolerance. The assumed explanation was that in the period when uranium is being excreted the kidney was manufacturing a great deal of citrate, which, because it formed a soluble complex with uranium, gave rise to an increased uranium excretion and to a decrease in toxicity.

13. THE ACTION OF URANIUM ON ENZYMES AND PROTEINS

There were several reasons why considerable importance was attached to the studies of reactions between uranium and proteins. In the first place, many heavy metals have been known to act as enzyme poisons, and it seemed possible that the action of uranium on the kidney tubules could be attributed to specific enzyme poisoning. It now is doubtful that this is the case.

In the second place, it seemed likely that proteins might be important in the transport of uranium in the blood. This has turned out to be true.

13.1 Reactions with Proteins, in Vitro. Many proteins combine firmly with U_6. Under the proper conditions such combination results in precipitation of the protein. The pH is important in the precipitation of proteins by U_6. As a rule, precipitation is greatest near the isoelectric point of the protein. The U_6-protein precipitate dissolves when strong uranium complexers like bicarbonate, citrate, or malate are added, owing to removal of U_6 from the protein by the complexer. One exception is known: U_6-histone precipitate does not dissolve in the presence of citrate. In some cases inorganic salt is needed if proteins are to be precipitated by U_6. A number of proteins have been classified into two groups depending on whether U_6 will precipitate them from solution with or without the addition of some salt.

In the presence of complexers, U_6 is a cation at low pH values and an anion at elevated pH values. Apparently the cationic form is the precipitating agent. The combination of U_6 with protein does not appear to cause an alteration of the protein, such as denaturation, provided the pH is always kept within limits tolerated by the protein in question. The lack of alteration is shown by several studies. Protein (egg albumin) precipitated by U_6 was found to be crystallizable after removal of the U_6 by dialysis in the presence of strong complexing agents; plasma proteins treated with U_6 were found to regain their normal mobilities after removal of the U_6 in the same manner as from the egg albumin; and enzymes treated with U_6 were found to regain activity after removal of U_6 in the same manner.

The U_6 appears to combine firmly with the carboxyl groups of proteins. There is good evidence that U_6 does not combine with SH or OH groups of proteins, and it appears to combine with the basic groups only in a loose ionic sense, if at all. A theory of precipitation has been worked out according to which each uranium ion combines with two carboxyls of different protein molecules, thus in effect joining the 2 molecules and providing the basis for a latticework type of precipitate.

The U_4 apparently acts on proteins in many ways very much like U_6. It precipitates proteins very strongly. Solution of the precipitates occurs in the presence of citrate or excess bicarbonate. Such manipulations may be carried out, at least in some cases, without apparent denaturation. The U_4 appears to differ from U_6 in having some affinity for SH groups.

13.2 Reaction with Enzymes, in Vitro. The study of the effect of U_6 on enzymes is complicated by its tendency to form complexes with the substances commonly used as substrates or buffers; such complexing weakens the action of U_6 on the enzyme. For this reason, buffers consisting of strong complexing anions, such as citrate, malate, or bicarbonate, must be avoided. Moreover it sometimes happens that an enzyme is sensitive to U_6 if the latter is added to the enzyme in the absence of substrate or buffer, but is insensitive if the U_6 is added to the buffer-substrate mixture before addition of the enzyme. Thus care must be taken to consider the experimental conditions critically before attempting to evaluate the results of an experiment on the inactivation of an enzyme by U_6 in vitro. The U_6 in relatively high concentration reversibly inhibits many enzymes to a slight or moderate degree, but in low concentration it is without effect on most of the enzymes studied.

Since there is a possibility that certain prosthetic groups or coenzymes might react with U_6 in such a way as to account for the inhibitory effect, enzymes containing a number of these groups have been studied. No reaction was observed between U_6 and SH groups. Hematin, flavine-adenine dinucleotide, and coenzyme I did not react with U_6 in such a way as to block their catalytic activities. However, U_6 appeared to form a complex with coenzyme I and may have combined with hematin through carboxyl groups.

It was found that phosphorylase, of which the prosthetic group is presumably adenylic acid, is strongly inhibited by U_6 in vitro. Since this inhibition was relieved by the addition of adenylic acid it seems probable that U_6 produces inhibition of this enzyme by combining with the adenylic acid, presumably through the phosphate group.

Table 4—Sensitivity of Enzymes in Vitro to U_6 and U_4

Adenosine triphosphatase*	(-)U_6
Adrenaline oxidase*	(-)U_6
d-Amino oxidase†	(-)U_6
Salivary amylase†	(-)U_6
Pancreatic amylase*	
Malt amylase*	
Arginase*	(-)U_6
Carbonic anhydrase (in presence of HCO_3^-)*	(-)U_6
Choline oxidase†	(-)U_6
Catalase‡	(-)U_6
Cytochrome oxidase*	(-)U_6
Esterase*	(-)U_6
Lactic dehydrogenase system containing diaphorase‡	(+)U_6
Pepsin†	(-)U_6; (+)U_4
Acid phosphatase†	(+)U_6
Alkaline phosphatase†	(-)U_6
Phosphoglucomutase*	(+)U_6; (++)U_4
Phosphorylase†	(++)U_6; (++)U_4
Succinic dehydrogenase*	(-)U_6
Thrombin†	(++)U_6
Urease*	(-)U_6; (+)U_4
Xanthine oxidase†	(-)U_6

(-)U_6: relatively insensitive to U_6.
(+)U_6: moderate sensitivity to U_6.
(++)U_6: quite sensitive to U_6.
(+)U_4: moderate sensitivity to U_4.
(++)U_4: quite sensitive to U_4.
* Partially purified enzyme or apoenzyme.
† Tissue brei or secretion used as enzyme preparation.
‡ Crystalline enzyme or apoenzyme used.

Urease, an SH enzyme used as a model in studying inhibition by heavy metals, is some 13,000 times more sensitive to silver than to uranium and some 400 times more sensitive to mercury than to uranium. However, in vitro the sensitivity of phosphorylase to U_6 is of the same order of magnitude as the sensitivity of urease to mercury. The latter sort of finding is uncommon and was encountered aside from phosphorylase in the case of thrombin only.

A considerable number of enzymes have been studied in vitro in regard to sensitivity to uranium, some in considerable detail. A list of these enzymes is appended in Table 4, with a comment as to whether or not the enzyme is sensitive to U_6 and in some cases to U_4. The action of U_4 on enzymes is in general similar to, but slightly more severe than, the action of U_6.

13.3 Enzyme Studies in Vivo. Because all tissue fluids are rich in anions that tend to complex with uranium, in vivo conditions are usually not favorable for inhibition of enzymes by U_6. This is well illustrated by the fact that phosphorylase does not appear to be sensitive to U_6 in vivo. An exception to this general statement could be made for any enzymes that may be located in the cell surfaces of the kidney tubules.

Table 5 — Effect of U_6 on Enzymes in Vivo

Enzyme	Effect on activity in various tissues			Species
	Kidney	Liver	Other	
d-Amino oxidase	Decrease	No change		Rat
Carbonic anhydrase	No change		Blood: no change	Rat
Catalase	Decrease in some cases			Rat, rabbit
Choline oxidase	Some decrease	Increase		Rat
Glucose oxidase	Enzyme not present in normal or poisoned animals	Decrease		Rat
Pepsin			Gastric contents: sometimes some decrease	Rat
Alkaline phosphatase	Decrease, especially in acute poisoning			Rat, rabbit
Phosphoglucomutase	No change	No change		Rat
Phosphorylase	No change	No change	Muscle: no change	Rat

A number of systems have been studied in which enzyme activities from tissues of animals poisoned by uranium were determined analytically. A list is given in Table 5.

The respiration of liver and kidney brei of rats showed a curious increase on about the second day following the administration of uranium, but later fell and was below normal on the fourth and fifth days.

Studies of the respiration of slices of certain tissues from normal and U_6-poisoned guinea pigs were made, especially as regards the oxidation of ascorbic acid by liver and kidney. Peculiarly enough, the oxidation of ascorbic acid by liver and kidney slices of normal guinea pigs is not changed by the addition of U_6, whereas the oxidation is increased in kidneys of scorbutic guinea pigs by the addition of U_6.

Since the kidneys of animals poisoned with U_6 show decreased activities (beginning a day or so after poisoning) of many enzymes that are relatively insensible to the metal in vitro, it must be concluded that these decreases in activity are only indirectly attributable to the action of U_6.

A plausible theory to explain the toxic action of uranium on the cells of the kidney tubules is that U_6 combines with proteins in the surfaces of these cells. If enzymes are involved directly, they probably are located in the cell surfaces so that the action of U_6 on these enzymes might tend to be quite general rather than specific.

14. CATALASURIA AS A SENSITIVE TEST FOR URANIUM POISONING

In the search for a sensitive method for the earliest detection of uranium damage to the kidney, Dounce proposed the measurement of urinary catalase activity. This suggestion has proved to be a happy one. The study of catalasuria has become accepted in the project laboratory as one of the best procedures in uranium toxicology.

In the first place, it was shown that the appearance of catalase activity in the urine is a sensitive reflection of acute damage to tubule cells. Red cells have a high activity, but as many as 400,000 red cells per milliliter of urine did not interfere with the sensitiveness of the test. Wills showed from catalase clearance studies that catalase of the plasma was not transmitted to the urine. Catalase activity reflected tubular cell damage.

Protein excretion in the urine is also a very sensitive indicator of uranium poisoning, and in some animals may be as sensitive as catalasuria, but protein is well known to be less specific for tubular damage. Phosphatase activity in the urine is also a valuable test but is probably less sensitive and certainly less repeatable than catalasuria or proteinuria. In a series of sacrifice tests it was shown that about 4 mg of uranyl ion per kilogram would evoke a catalasuria comparable to that produced by a little over 1 mg of mercuric ion per kilogram or 1 mg of arsenic as mapharsen per kilogram. In survival experiments, however, judging by the catalasuria test, U_6 was found to be three to five times more poisonous than mercury.

A portable machine for the estimation of catalase has been designed and built. The conditions under which tests should be made have been established, and it has been shown that occasional check determinations using standard catalase solutions are a wise precaution.

In tests on animals, catalase response in uranium poisoning has been demonstrated in dogs, cats, rabbits, and rats. Elaborate precautions have been found necessary in the collection of urine samples from animals in order to obtain reliable information. In both rabbits and dogs it has been demonstrated that 0.01 mg of uranyl ion per kilogram of body weight will usually produce a detectable catalasuria. Repeated doses eventually evoke lesser responses. Detailed studies of rabbits that received 15 doses during a 100-day period and of dogs that received 12 doses in 200 days have shown a repetition of response of catalasuria.

In animals exposed to uranium compounds by inhalation of dusty atmospheres of nitrate or of the oxyfluoride, 2.0 to 0.5 mg/cu m produced catalasuria in rabbits and dogs, whereas nitrate at a dust concentration of 0.3 mg/cu m resulted in some indication of a catalasuria in rabbits and a minimal catalasuria in dogs.

In a series of studies on rabbits injected with soluble uranium compounds, the urinary catalase activity and protein content was followed as was the blood NPN and the histopathology of the kidney. It was shown that it was possible to find a dose that would be followed by an increase in catalase activity of the urine in the absence of positive changes in the other criteria listed.

15. THE MECHANISM OF ACTION OF URANIUM COMPOUNDS IN THE ANIMAL BODY

A considerable part of the intricate story of uranium poisoning has been discovered and woven into a satisfactory explanation of the changes that have been observed following exposure to uranium compounds. There is general agreement that the primary effect of uranium is on the kidney. A great deal of attention has been paid to the changes in this organ. Many other effects of uranium on the body have been observed and measured. For the most part, these are now considered to be secondary effects and consequences of the primary injury to the kidney.

The absorption of uranium compounds via a number of routes has been tested as sources of uranium poisoning. Uranium compounds are not readily absorbed through the skin. However, it has been amply demonstrated in rabbits that sufficient amounts to produce severe poisoning may be absorbed. The gastrointestinal tract also is not highly permeable to uranium, and much larger doses may be tolerated in this wise than via the lung or parenteral routes. It is probable that all the uranium compounds studied have been converted into U_6 before absorption, with the exception of the U_4 in solution administered intravenously.

The U_6 in the blood is carried in two principal forms: (1) complexed with bicarbonate and (2) complexed with protein. Most of the U_6 is probably a bicarbonate complex. Most of the U_4 is probably a protein complex, or a colloidal oxide stabilized by protein. The U_6 is rapidly lost from the blood, principally into bone and into the kidneys. Eventually a large proportion appears in the urine. The U_4 leaves the blood somewhat more slowly, remains in higher concentrations for longer periods in the soft tissues, enters the kidneys in smaller amounts, and is excreted in lesser proportion in the urine. In both U_6 and U_4, storage in the bone constitutes the main permanent residuum of a dose of uranium. Uranium is adsorbed on bone very tenaciously.

The kidney is the principal site of uranium injury. The U_6 taken by the blood to the kidney passes as the bicarbonate complex into the glomerular filtrate. As the pH of the urine becomes more acid, and as bicarbonate is resorbed by tubule epithelial cells in the distal portion of the proximal convoluted tubule, the amount of bicarbonate becomes insufficient to keep the uranyl ion as a soluble complex, and the uranium combines with the protein wall of the cell surface. The cell, if sufficiently damaged by combination with uranium, dies, disintegrates, and is sloughed off. If the animal recovers, regenerated epithelial cells again cover the inner surface of the tubule, and apparently most of the functions are regained.

In the meantime, renal-function tests show cell deficiencies. Inulin clearances give no indication of decrease in filtration in the early stages of poisoning, but later fall as kidney function becomes minimal and the animal anuric. Chloride clearances, which indicate the ability of certain epithelial cells to resorb chlorides, rise, indicating failure in this normal function. Diodrast clearances, which show the excretory power of certain tubular cells, fall, showing the failure of this function. Catalase appears in the urine, and in severe poisoning phosphatase also appears. These urinary enzymes have their origin in the cells of the tubule. Protein also appears in the urine. The origin is partly from the plasma and partly from necrotic cells.

A marked increase in the excretion of urinary citric acid has been observed. This rises to a peak a few days after the administration of uranium. Following a return to normal for a week or more, a second prolonged peak has been found. Uranium administered during the second peak is tolerated better than the original dose. An animal repeatedly treated in this fashion is able to withstand relatively excessive doses of uranium that would have almost certainly been fatal in an untreated animal. There is some evidence that the regenerated tubule cell has an altered behavior and is able in some fashion, perhaps related to the citrate excretion, to resist the destructive effect of uranium.

Based on the knowledge of the mechanism of uranium poisoning, it is possible to suggest various therapeutic and prophylactic measures. A large number of possible therapeutic agents have been tested, most of which were found to be without any effect; a few were shown to be harmful. Measures that would promote the excretion of bicarbonate and possibly of citrate seem to be helpful. The administration of bicarbonate thus far has been found the best therapeutic agent.

Many secondary effects have been described on the metabolism of poisoned animals. These include an interference with carbohydrate metabolism resembling somewhat a diabetic response to the sugar tolerance test. Changes in the unsaturation of certain fatty acids of liver and kidney have been described. Profound changes in nitrogen metabolism, particularly the azotemia associated with acute kidney damage, have been shown to be a reliable index of severe poisoning. Some interference with vitamin C metabolism has been suggested. Phenol and phosphorous metabolisms have been studied. Water balance is altered. The over-all metabolic effect in fatally poisoned animals is reflected in a premortal fall in body temperature. A great deal of work has been done to find what enzymes or enzyme systems are influenced by concentrations of uranium such as may be obtained in the body. It is unlikely that significant enzyme poisoning is caused by small or moderate doses of uranium in any site in the body with the possible exception of the kidney tubules.

Part II. Fluorine and Fluoride Gases

Several important discoveries have come from the studies of the inhalation toxicity of fluorine compounds. Elemental fluorine, and hydrogen fluoride were studied. One of the hazards of handling high-pressure fluorine (F_2) is the danger of burns. The method of treating heat burns is quite different from that of HF burns; consequently it was important to discover that the F_2 burn apparently is predominantly a heat burn.

Very high concentrations of F_2 were inhaled by rabbits, guinea pigs, rats, and mice for brief periods. Starting with exposure to 10,000 ppm F_2 for 5 min, the concentration was decreased to 200 ppm, and the duration of exposure increased to 3 hr. Every animal in these experiments died as a result of the exposure. A further decrease in concentration to 100 ppm for 7 hr produced roughly a 60 per cent mortality.

In 30-day exposure studies dogs, rabbits, rats, mice, and hamsters in various groups were exposed to atmospheres containing 25, 8, 3, and 0.8 mg F_2/cu m. The concentrations that killed roughly half the animals during the 30-day period were as follows: rats and guinea pigs, 25 to 8 mg/cu m; rabbits and dogs, 8 to 3 mg/cu m. The dog and rabbit were susceptible to F_2 poisoning; the rat was resistant.

Thirty-day exposures were conducted in which rabbits, dogs, guinea pigs, rats, and mice were exposed to two concentrations of HF, namely, 25 and 7 mg/cu m. The concentrations that would kill half the animals in 30 days were greater than 25 mg/cu m for the rabbit, dog, and guinea pig, and were 25 and 7 mg/cu m for the rat and mouse. The dog and rabbit were resistant to HF exposure, and the rat was susceptible.

Groups comprising six rats and six mice were exposed to various concentrations of OF_2 for short-time periods. All the animals inhaling 10 to 0.5 ppm died. In a 30-day exposure study at a concentration of 0.1 ppm, there was no mortality and no detectable toxic response.

A safe concentration for HF and F_2, under the conditions used would appear to be as follows: HF, 7 mg/cu m; F_2, 1 mg/cu m.

An enzymatic method was used for the analysis of certain samples of chamber air in which HF or UF_6 had been added.

The LD_{50} for a 24-hr period following the administration of NaF solutions intraperitoneally was shown to be of the order of 47 mg/kg in 100- to 200-g rats and 24 mg/kg in 200- to 300-g rats.

PART I

THE PHARMACOLOGY AND TOXICOLOGY OF URANIUM COMPOUNDS

Chapter 1

THE CHEMISTRY OF URANIUM COMPOUNDS

By Alexander L. Dounce and John F. Flagg*

This chapter will deal with the principal sources and compounds of uranium that are of interest in connection with toxicological studies. No effort has been made to include all uranium compounds, or to describe all known reactions of those that are included. The information given is that considered to be of most potential value in matters relating to the toxicology of uranium-containing materials.

The first section of the chapter deals with general reactions and properties. In a later section the various important uranium complexes that are encountered in biological systems are discussed.

1. PREPARATION AND PROPERTIES OF URANIUM COMPOUNDS

By John F. Flagg

1.1 Uranium Ores. The principal ores of uranium are pitchblende and carnotite. Pitchblende is a dark, bluish-black mineral, containing uranium in the form of oxides UO_2 and UO_3 in such ratio as corresponds rather closely to the formula U_3O_8. A low-grade pitchblende may contain approximately 10 per cent uranium oxides, while the high-grade ore may contain as much as 70 per cent. Radium and lead, decomposition products of the uranium, are also present in the ores, together with such elements as aluminum, arsenic, calcium, iron, magnesium, manganese, and many others. A typical analysis of high-grade pitchblende is given in Table 1.1. For each gram of uranium in old, undisturbed ores there is 3.40×10^{-7} g of radium and roughly 0.1 g of lead (RaG).

Carnotite is a canary-yellow mineral containing potassium, uranium, and vanadium; the proportions are approximately those of the formula $K_2O.2UO_3.V_2O_5.3H_2O$. Lead and radium are also present, together with traces of barium, calcium, and other metals.

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*Work done by Alexander L. Dounce, John F. Flagg, Paul Fanta, T/3 G. H. Tishkoff, and Tien Ho Lan, with the assistance of Marian Kaley.

Less important sources of uranium are the minerals autunite, $Ca(UO_2)_2(PO_4)_2.8H_2O$, and torbernite, $Cu(UO_2)_2.(PO_4)_2.8H_2O$.

Table 1.1 — Analysis of High-grade Uranium Ore*

Constituent	Per cent	Constituent	Per cent
U_3O_8	47.26	Fe_2O_3	0.94
UO_2	20.94	Al_2O_3	1.36
He	0.12	TiO_3	0.17
SO_3	1.27	CaO	2.22
MoO_3	0.74	MgO	2.27
PbO	6.09	Na_2O	0.86
NiO	1.04	CO_2	0.57
CoO	0.43	H_2O (1000°C)	2.50
SiO_2	10.53	Rare-earth oxide	0.13
		Total	99.44

Reported below 0.10 per cent: B, Cd, P_2O_5, Cl, Os, Pd, Pt, Se, As, Mn, O, CuO.

*African ores; Lot AAA; dried at 110°C; analysis from industrial source.

Uranium may be extracted from most ores by treatment with nitric acid or with a mixture of nitric and hydrochloric acids. Nitric acid will dissolve most constituents in pitchblende with the exception of siliceous material. Lead and radium may be precipitated together as sulfates from the nitric acid solution, leaving uranium as soluble uranyl nitrate. Under proper conditions the uranium is then extracted from the nitric acid solution with ether.

1.2 Uranium Compounds.* In the dry state, uranium forms compounds in which the valence of the element is 3, 4, 5, or 6. In aqueous solution, however, compounds of trivalent and pentavalent uranium are unstable; the trivalent form is readily oxidized by either dissolved oxygen or by water itself, and the pentavalent form disproportionates into U_4 and U_6. Hexavalent uranium is the most stable form in aqueous solution, existing as the oxygenated cation UO_2^{++} in acid solutions. The common uranyl salts, solid and in solution, are yellow in color; solutions containing the uranyl ion exhibit a green-yellow fluorescence. Uranous salts and their solutions are deep green, but solutions of trivalent uranium salts are of a reddish color.

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*In the following pages the term U_6 is used as a general term for hexavalent uranium and does not refer to 6 atoms of uranium. Similarly, U_4 refers to tetravalent uranium, U_3 to trivalent, and U_5 to pentavalent.

Important uranium compounds include the oxides and halides, which, with a few others, will be described in the following sections. In Table 1.2 are data indicating the degree of purity of some typical compounds.

Table 1.2 — Uranium Content of Certain Industrially Important Uranium Materials

Uranium dusts	Analysis		
	Uranium content		Probable major impurities
	Per cent found*	Per cent calculated	
Oxides:			
UO_2	88.17	88.15	U_3O_8, UH_3
UO_3	81.51	83.22	UO_1
$UO_4.3H_2O$	66.7	66.8	$UO_2(NO_3)_2$
U_3O_8	83.6	84.8	
Fluorides:			
UF_4	78.4	75.8	UO_2F_2, UO_2
UF_6		66.6	HF not more than 3% and probably much less following treatment for use.
UO_2F_2	76.8	77.3	Na_2UO_4
Diuranates:			
$Na_2U_2O_7.1\frac{1}{2}H_2O$	79.1	74.2	
$(NH_4)_2U_2O_7.4H_2O$	70.8	71.3	
Chloride:			
UCl_4	60.8	62.6	UH_3, UO_2Cl_2 (?)
Nitrate:			
$UO_2(NO_3)_2.4H_2O$†	53.0	51.0	
High-grade ore	59.9		PbO

*Average of at least three closely agreeing values obtained by the analytical group of the Inhalation Section employing a modification of the ferrocyanide acetic acid method.

†Dust desiccated to constant weight in vacuo to approximate water content of 4 moles per mole of nitrate. Samples before desiccation showed variable uranium contents ranging from 47 to 51 per cent.

(a) Uranium Tetrachloride. This substance may be prepared by any of several reactions (Eqs. 1 to 3).

$$\left.\begin{array}{l} UH_3 + 3HCl \xrightarrow{250^\circ} UCl_3 + 3H_2 \\ 2UCl_3 + Cl_2 \rightarrow 2UCl_4 \end{array}\right\} \qquad (1)$$

$$\left.\begin{aligned}&2UH_3 + 3Cl_2 \rightarrow 2UCl_3 + 3H_2\\&2UCl_3 + Cl_2 \rightarrow 2UCl_4\end{aligned}\right\} \qquad (2)$$

$$UO_3 + CCl_4 \rightarrow UCl_4 + \text{by-products, including C, } COCl_2 \qquad (3)$$

Uranium tetrachloride crystallizes in green needles, the melting point of which is 590°C. The salt combines readily with water at room temperature, forming first a hydrate, $UCl_4.2H_2O$, which is converted to $UOCl_2.xH_2O$ as the amount of water is increased.

(b) Uranium Tetrafluoride. Uranium tetrafluoride is a green powder, moderately stable when exposed to air, and insoluble in water and dilute acids. It is prepared in the dry way (Eqs. 4 to 7) or in the wet way (Eq. 8) by reactions employing hydrogen fluoride. It is also obtained by the reduction of uranium hexafluoride (Eqs. 16 and 17).

$$2UH_3 + 8HF \rightarrow 2UF_4 + 7H_2 \qquad (4)$$

$$U + 4HF \rightarrow UF_4 + 2H_2 \qquad (5)$$

$$UO_2 + 4HF \rightarrow UF_4 + 2H_2O \qquad (6)$$

$$\left.\begin{aligned}&Na_2UO_4 + 4HF \rightarrow UO_2F_2 + 2NaF + 2H_2O\\&UO_2F_2 + 4HF + SnCl_2 \rightarrow UF_4 + SnCl_2F_2 + 2H_2O\end{aligned}\right\} \qquad (7)$$

The compound melts at 960 to 965°C. Heating in air converts uranium tetrafluoride to the oxide U_3O_8. Treatment with sodium hydroxide produces insoluble $U(OH)_4$, and ammonia and hydrogen peroxide also decompose the compound, forming $(NH_4)_2U_2O_7$. Active metals such as potassium or magnesium reduce uranium tetrafluoride to metallic uranium.

$$UF_4 + 2Mg \rightarrow U + 2MgF_2 \qquad (8)$$

The compound may contain up to several per cent of uranyl fluoride, UO_2F_2, as an impurity. Lesser amounts of heavy metals, such as iron and copper, are also encountered in commercial preparations.

(c) Uranium Dioxide. This substance, a dark-brown powder commonly referred to as "brown oxide," is prepared by the action of water on uranium hydride (Eq. 9) or by the reduction of U_3O_8 (Eq. 10).

$$2UH_3 + 4H_2O \rightarrow 2UO_2 + 7H_2 \qquad (9)$$

$$U_3O_8 + 2H_2 \rightarrow 3UO_2 + 2H_2O \qquad (10)$$

The oxide is insoluble in water and dilute hydrochloric or sulfuric acid but dissolves in concentrated hydrochloric acid to form UCl_4 (Eq. 11).

$$UO_2 + 4HCl \rightarrow UCl_4 + 2H_2O \tag{11}$$

Nitric acid reacts with the oxide to form uranyl nitrate (Eq. 12), as does a mixture of nitric and hydrochloric acids.

$$3UO_2 + 8HNO_3 \rightarrow 3UO_2(NO_3)_2 + 2NO + 4H_2O \tag{12}$$

Uranium dioxide is highly refractory, melting in the region of 2200 to 2600°C.

(d) Uranium Hexafluoride. This compound may be obtained either by the action of fluorine on uranium pentachloride (Eq. 13) or by treating uranium or uranium carbide with fluorine in the presence of chlorine (Eq. 14).

$$2UCl_5 + 5F_2 \rightarrow UF_4 + UF_6 + 5Cl_2 \tag{13}$$

$$U + 3F_2 \rightarrow UF_6 \tag{14}$$

Another method for the preparation of uranium hexafluoride involves the action of fluorine on uranium tetrafluoride (Eq. 15).

$$UF_4 + F_2 \rightarrow UF_6 \tag{15}$$

The hexafluoride boils at 56.4°C. It is an extremely corrosive substance and acts as a fluorinating agent (Eqs. 16 and 17).

$$UF_6 + H_2 \rightarrow UF_4 + H_2F_2 \tag{16}$$

$$UF_6 + Fe \rightarrow UF_4 + FeF_2 \tag{17}$$

It is readily hydrolyzed by water, forming uranyl fluoride (Eq. 18).

$$UF_6 + 2H_2O \rightarrow UO_2F_2 + 2H_2F_2 \tag{18}$$

(e) Uranium Trioxide. This compound, often referred to as the "yellow oxide," is obtained by calcining the peroxide in a stream of oxygen (Eq. 19).

$$2UO_4.2H_2O \rightarrow 2UO_3 + 4H_2O + O_2 \tag{19}$$

The oxide is hygroscopic and dissolves readily in acids, forming the corresponding uranyl salts. Upon heating at 600°C the trioxide is converted to U_3O_8. Carbon monoxide and chlorine react with UO_3 at elevated temperatures to form uranyl chloride and uranium tetrachloride.

(f) Uranium Tetroxide (Peroxide). This yellow peroxide is precipitated from acid solutions of uranyl salts by hydrogen peroxide (Eq. 20).

$$UO_2(NO_3)_2 + H_2O_2 + 2H_2O \rightarrow UO_4.2H_2O + 2HNO_3 \qquad (20)$$

Water appears to be an essential part of the molecule, and the compound is sometimes referred to as "peruranic acid," H_4UO_6. The compound is soluble in alkalies, forming peruranates. Heating causes decomposition to UO_3 or U_3O_8 (Eq. 19).

(g) Uranium Tritaoctoxide. Heating most uranium salts in air at temperatures of about 1000°C results in the formation of this oxide. As mentioned in Sec. 1.1, it is a principal constituent of pitchblende. The oxide is dark green to black in color, hence the designation of "black oxide." It is insoluble in water but dissolves readily in nitric or concentrated sulfuric acid with the formation of the corresponding uranyl compounds. Aqueous hydrofluoric acid with U_3O_8 forms uranyl fluoride and uranium tetrafluoride, but hydrochloric acid is practically without action upon it. Both hydrogen and active metals will reduce the oxide (Eqs. 21 and 22).

$$U_3O_8 + 2H_2 \rightarrow 3UO_2 + 2H_2O \qquad (21)$$

$$U_3O_8 + 8Ca \rightarrow 3U + 8CaO \qquad (22)$$

The oxide is thought to be composed of two molecules of UO_3 and one of UO_2.

(h) Uranyl Acetate. When uranyl nitrate or uranium trioxide is treated with acetic acid, uranyl acetate is formed (Eqs. 23 and 24).

$$UO_2(NO_3)_2.6H_2O + 2C_2H_4O_2 \rightarrow UO_2(C_2H_3O_2)_2.2H_2O + 2HNO_3 + 4H_2O \qquad (23)$$

$$UO_3.H_2O + 2C_2H_4O_2 \rightarrow UO_2(C_2H_3O_2)_2.2H_2O \qquad (24)$$

The compound exists as yellow prisms, which lose water at temperatures of 110°C and higher. It is soluble in water, alcohol, and pyridine but insoluble in ether and chloroform. Complex acetates are well known; these are discussed in Sec. 2.1.

(i) Uranyl Fluoride. This compound may be prepared by the action of uranium hexafluoride on water (Eq. 18). Uranyl fluoride is readily soluble in water and in methanol, a property employed in separating it from the tetrafluoride. Strong ignition converts uranyl fluoride to U_3O_8.

(j) Uranyl Nitrate. The action of nitric acid upon any of the oxides UO_2, UO_3, or U_3O_8 results in the formation of uranyl nitrate (Eq. 25).

$$U_3O_8 + 8HNO_3 \rightarrow 3UO_2(NO_3)_2 + 2NO_2 + 4H_2O \qquad (25)$$

The salt is yellow as a solid and in solution. It is readily soluble in numerous organic solvents as well as in water and may be extracted quantitatively by ether from aqueous solutions saturated with metallic nitrates. Such metallic nitrates commonly used as salting agents are calcium nitrate, copper nitrate, aluminum nitrate, sodium nitrate, and ammonium nitrate, although the nitrates of many other metals are effective.

Uranyl nitrate, in common with other uranyl salts, reacts with alkalies to form insoluble uranates or diuranates such as Na_2UO_4, $Na_2U_2O_7$, $(NH_4)_2U_2O_7$, etc. These compounds are soluble in acids and are also dissolved by complexing agents such as the carbonate or bicarbonate ion (see Sec. 2.1).

Upon being heated, uranyl nitrate first loses water, in successive stages, and then finally is converted into U_3O_8.

(k) Miscellaneous Compounds. A few other compounds of uranium are worth brief mention. The hydride, important as a starting material for the preparation of other uranium compounds, is prepared by direct combination of the elements at elevated temperature. It is pyrophoric. Three high-melting carbides, UC, U_2C_3, and UC_2, and two nitrides, UN and $UN_{1.5-2}$, are known.

2. CHEMISTRY OF URANIUM COMPOUNDS WITH PARTICULAR REFERENCE TO BIOCHEMICAL AND TOXICOLOGICAL STUDIES

By A. L. Dounce, G. H. Tishkoff, Paul Fanta, and Tien Ho Lan*

2.1 Chemistry of U_6-complex Formation. From the biochemical and toxicological viewpoints the principal interest in U_6 is concentrated on the formation of complexes of the uranyl ion with various inorganic and organic anions in solution at pH values near 7.0. This is because U_6 is only slightly soluble at pH values of 7.0 or higher in the absence of complexing anions but has a very pronounced tendency to form soluble complexes with a number of anions present in body fluids. The behavior of U_6 in the presence and absence of complexers may be demonstrated by adding 0.1N NaOH slowly to aqueous 0.5 per cent solution of uranyl acetate and uranyl nitrate. If the NaOH is added carefully, the pH of the acetate solution can be brought up to 8.0 or higher without the appearance of a precipitate, whereas a hydrous oxide of U_6 commences to precipitate from the uranyl nitrate solution when the pH has reached 4.0. The reason for the difference in behavior of the two solutions may be that the acetate anion is a complexer for the uranyl ion, whereas the nitrate anion has little or no tendency to form complexes with the uranyl ion in aqueous solution, so that the ion is free to form the insoluble oxide as the pH is raised. Complexes of U_6 apparently must be broken down before oxide formation can occur.

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*Section 2.3c on oxidation of U_4 by molecular oxygen is by Tien Ho Lan.

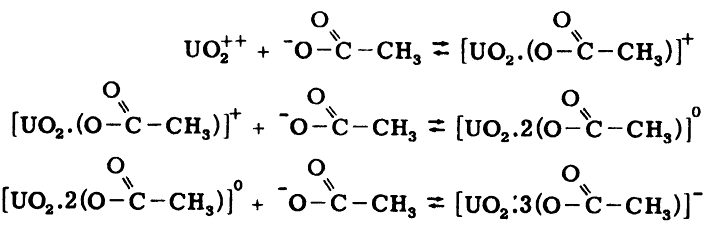
Included in the list of complexers for the uranyl ion are the following classes of substances:

1. Certain inorganic ions (phosphate, bicarbonate, carbonate).
2. Organic phosphates (glyceryl phosphate, glucose-1-phosphate).
3. Monocarboxylic organic acid anions (acetate, pyruvate, lactate).
4. Special complex organic acids (citrate, malate).
5. Proteinate.

The examples given of each class of substance are those which have been studied in the project laboratory.

At present the exact chemical constitution of the complexes cannot be specified. However, it can be said that the complexes of the uranyl ion themselves usually are anions, although sometimes they appear to be neutral in charge. None of the complexes thus far directly investigated has been cationic in nature, but it seems probable from theoretical considerations that in certain cases small amounts of cationic complexes may exist.

The following equations, which are more or less hypothetical, will illustrate how complexes of U_6 with acetate might be formed:



$$UO_2^{++} + {}^-O-\overset{O}{\overset{\|}{C}}-CH_3 \rightleftharpoons [UO_2.(O-\overset{O}{\overset{\|}{C}}-CH_3)]^+$$

$$[UO_2.(O-\overset{O}{\overset{\|}{C}}-CH_3)]^+ + {}^-O-\overset{O}{\overset{\|}{C}}-CH_3 \rightleftharpoons [UO_2.2(O-\overset{O}{\overset{\|}{C}}-CH_3)]^0$$

$$[UO_2.2(O-\overset{O}{\overset{\|}{C}}-CH_3)]^0 + {}^-O-\overset{O}{\overset{\|}{C}}-CH_3 \rightleftharpoons [UO_2{:}3(O-\overset{O}{\overset{\|}{C}}-CH_3)]^-$$

In these equations the nature of the bonding between the uranyl group and the acetate group has not been specified. Furthermore the formal charges assigned to the complexes as a result of algebraic addition of the charges on the uranyl ion and the acetate groups are not necessarily correct, since the complexes might conceivably take up one or more molecules of water and ionize negatively as the result of dissociation of hydrogen ions. In the case of complexing of UO_2^{++} with anions of dicarboxylic or tricarboxylic acids, it is obvious that the complexes formed might have considerably more negative charges than in the case of a monocarboxylic acid like acetic acid. However, in the case of citric acid, judging from the anionic mobility of the uranium (Fig. 1.1), this does not appear to be the case, probably because only one citrate is complexed per atom of uranium.

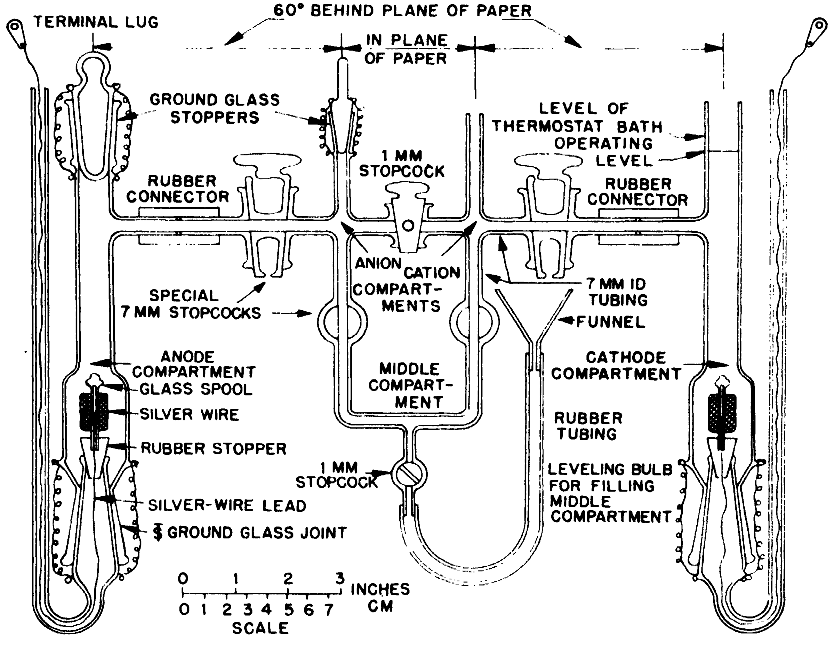


Fig. 1.1 — Apparatus for determining electrophoretic mobility of uranium complexes.[1]

Polarographic work has demonstrated the existence of complexes of UO_2^{++} with two and with three acetate groups as postulated in the foregoing equations. The existence of the uranyl monoacetate complex has not been demonstrated directly, nor has the magnitude of the charges on the complexes been determined by experimental procedure.

The methods used in the project laboratory for investigating the action of complexing substances for the uranyl ion involved the use of (1) electrical transport or electrophoresis studies, (2) the polarograph or dropping-mercury electrode, (3) the spectrophotometer, (4) ferrocyanide as a color reagent to measure the free UO_2^{++} ion present,

(5) titration data (especially applicable in the case of protein), (6) the ability of a given complexer to dissolve protein (usually egg albumin) that has been precipitated by U_6.

These methods will now be considered in the order given above, some in considerably more detail than others.

(a) Use of Electrical Transport Studies or Electrophoresis to Investigate Complexers of U_6. This method, although only semiquantitative in nature, has proved to be generally applicable and of considerable importance in gaining information about complexes of U_6. The first study of the electrophoretic transport of U_6 in acid solution and in neutral solution in the presence of acetate was carried out by Alling, using the Tiselius apparatus. In acid solution the U_6 was found to migrate toward the cathode, and in neutral solution in the presence of acetate it migrated toward the anode. Since the Tiselius apparatus was not available for extensive work, a special cell, which was connected to silver-silver chloride electrodes, was used (see Fig. 1.1). The electrophoresis was carried out at +2°C in order to minimize convection, which would upset the boundaries formed between the buffer and the U_6 buffer solutions. The anodic and cathodic sections of the cell were washed out and analyzed for U_6 following the electrophoresis, using the ferrocyanide method of analysis (see Chap. 2). By comparing the anodic and cathodic migration velocities of U_6, information could be gained concerning the relative amounts of free uranyl cation and complex anion of U_6 present under various conditions of pH and concentration of complexer. The possibility of occurrence of an appreciable amount of uncomplexed U_6 anion was more or less ruled out, since under conditions where anions of U_6 were found experimentally to be present (i.e., from pH 5 to 8), the U_6 was found to be highly insoluble in the absence of added complexing agent, as already has been mentioned. In other words, some complexing agent must be present to prevent the precipitation of the U_6 as oxide when the pH is raised above 5.0. In the experiments to be reported, no tendency whatsoever for precipitate formation in the solutions was observed.

The experiments using electrophoresis were of a semiquantitative nature as has been stated. The amounts of U_6 found by analysis in the cathode and anode compartments were expressed in arbitrary units. These units probably are not proportional to the amounts of cation and anion present in the solution under the given conditions, since the migration velocities of U_6 cation and U_6 complex anion no doubt are different in magnitude as well as in sign. Moreover, estimation of the amount of uncharged complex present has not been taken into account, except in cases where both cathodic and anodic migration are almost zero. However, the method is useful in that it shows under what conditions the U_6 is mainly cation, mainly complex anion, or mainly uncharged complex. From a quantitative standpoint, intermediate states must be dealt with by other means.

The electrophoretic behavior of U_6 in the presence of 0.05M acetate buffer and 0.05M KCl is shown in Fig. 1.2. This experiment and many others that follow were carried out before it was realized that there is little or no direct effect of pH on the distribution of U_6 between UO_2^{++} cation and complex U_6 anion. The effect of pH that apparently is demonstrated in this figure is indirect. Since the concentration of acetate plus that of un-ionized acetic acid was always kept 0.05M, it follows from an elementary consideration of a buffer system that, as the pH varies, the ratio of acetate to un-ionized acetic acid must vary continuously within the operating range of the buffer from low ratios at low pH values to high ratios at high pH values. It has been found that only the acetate anion, not the un-ionized acetic acid, can complex U_6 and that the ratio of complexed U_6 to UO_2^{++} cation increases as the

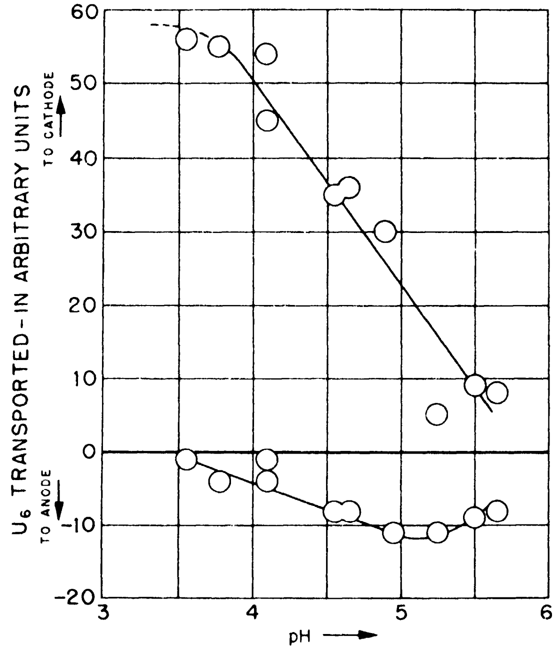
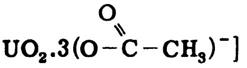


Fig. 1.2—Electrical transport of 0.004M uranyl acetate with 0.05M potassium chloride in 0.05M acetate buffer.

ratio of acetate anion to total U_6 increases. In other words, increasing the amount of acetate anion present increases the amount of U_6 acetate present and vice versa by the mass-law effect, as can be seen from the three equations already given earlier to describe the complexing action of acetate on U_6. Hence at low pH values where there is little acetate present there will be little U_6-acetate complex present, and at high pH values where much acetate is present, most of the U_6 will be in the form of an acetate complex.

In Fig. 1.2 there is no pH at which the U_6 appears to be mainly in the form of an uncharged complex, although some uncharged complex presumably is present in the neighborhood of pH 5.0.

In order to show that the ratio of acetate complex of U_6 [presumably  $UO_2.3(O-\overset{O}{C}-CH_3)^-$] to uncomplexed UO_2^{++} cation is high and constant at a fixed and relatively high acetate concentration regardless of pH, an experiment was carried out, the results of which are shown in Fig. 1.3. In this case the acetate anion concentration was always kept 0.1M, and the pH was varied by varying the amount of acetic acid present. The small percentage of ionization of acetic acid in the presence of sodium acetate can be neglected. It can be seen that the amount of UO_2^{++} present is relatively small and that the amount of U_6 acetate complex (which is negatively charged) is constant from pH 4 to pH 5.3.

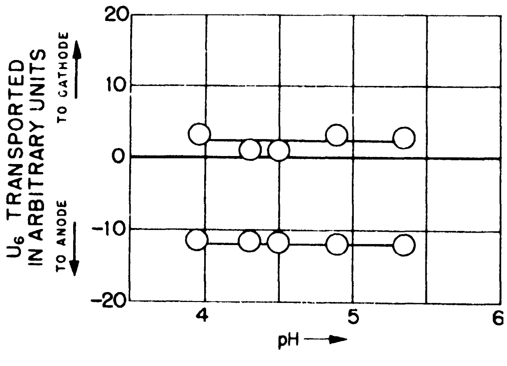


Fig. 1.3—Electrical transport of 0.004M uranyl nitrate hexahydrate with 0.1M potassium chloride and 0.1M sodium acetate. Different pH values are obtained by using various concentrations of acetic acid.

Finally, in Fig. 1.4 are shown the results of an experiment in which the concentration of acetate anion is varied over a wide range, while the pH is kept relatively constant (4.2 to 4.6). The effect of the concentration of acetate anion is directly demonstrated. This figure is of interest in that it demonstrates the presence of an uncharged acetate complex of U_6 at an acetate concentration of 0.025M. From polarographic studies it has been demonstrated that this complex is a biacetate to which the formula $UO_2.2(O-\overset{\overset{O}{\|}}{C}-CH_3)^0$ already has been tentatively assigned.

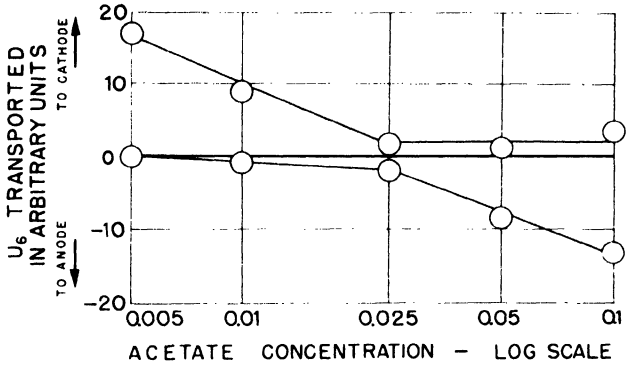


Fig. 1.4—Electrical transport of 0.004M uranyl nitrate hexahydrate with 0.1M potassium chloride at nearly constant pH (4.2 to 4.6) in 1:1 sodium acetate-acetic acid buffer.

It will be noted under polarographic studies that the change from the U_6 biacetate to the U_6 triacetate occurs at 0.05M acetate instead of 0.025M acetate as determined by means of electrophoresis. The most obvious explanation for this discrepancy would be that the temperature was different in the two experiments. The polarographic work was carried out at 25°C, whereas the electrophoresis was carried out at 2°C. Differences in uranium concentration apparently cannot explain the discrepancy as will be noted by consideration of the platinum electrode work to be reported in Sec. 2.3.

The experiments on the formation of acetate complexes of U_6 serve as models for electrophoretic studies of complex formation between U_6 and complexing substances. Unfortunately, owing to lack of time, it has been impossible to study other U_6 complexes in as great detail. In general, the least useful type of experiment was carried out, namely, the effect of pH on the distribution of U_6 between the uranyl cation and the complex anion or neutral complex. This type of experiment was carried out because the true nature of the so-called "pH effect" was not understood for some time. However, some idea of the relative complexing power of the various materials tested can be obtained from the results of the experiments.

In Fig. 1.5 is shown the behavior of U_6 in lactate buffer. This figure should be compared with the corresponding figure for acetate buffer (Fig. 1.2). No particular comment is necessary, except that apparently much uncharged U_6-lactate complex is present at pH 4.1, corresponding to a lactate anion concentration of 0.033M; moreover, anionic U_6-lactate complex is not demonstrated at any pH in high concentration, presumably because the lactate concentration is never very high.

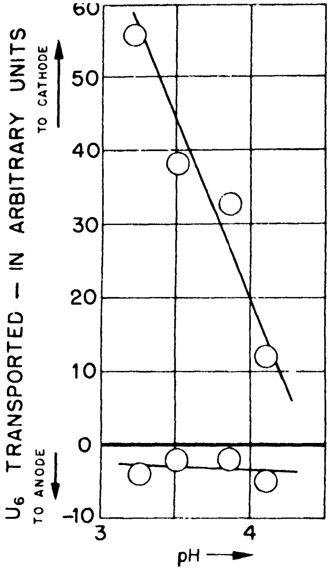


Fig. 1.5—Electrical transport of 0.004M uranyl nitrate hexahydrate with 0.05M potassium chloride in 0.05M lactate buffer.

In Fig. 1.6 is shown the behavior of U_6 in pyruvate buffer. There appears to be a high amount of uncharged complex present at pH 3.0, corresponding to a pyruvate anion concentration of 0.075M. At pH 3.7, corresponding to pyruvate anion concentration of 0.09M, the U_6 appears to be chiefly in the form of complex anion.

The electrophoretic behavior of U_6 in maleic acid-monosodium maleate buffer is shown in Fig. 1.7, and the behavior in monosodium maleate-disodium maleate buffer is shown in Fig. 1.8. In the case of the maleic acid-monosodium maleate buffer, it appears that no anionic U_6 complex is formed. At pH 2.6 corresponding to a monosodium maleate concentration of 0.038M, there is considerable uncharged complex, since the cationic migration velocity is less than one-third of its maximal value at pH 1.8, and the anionic migration velocity is still negligible.

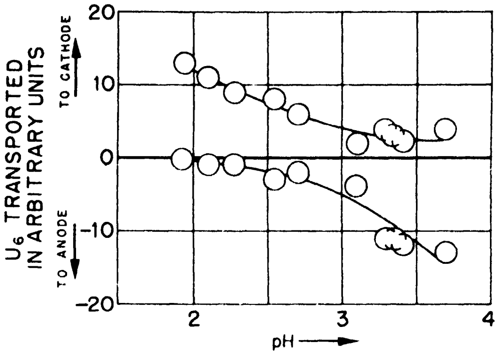


Fig. 1.6—Electrical transport of 0.004M uranyl nitrate hexahydrate with 0.05M potassium chloride in 0.1M pyruvate buffer.

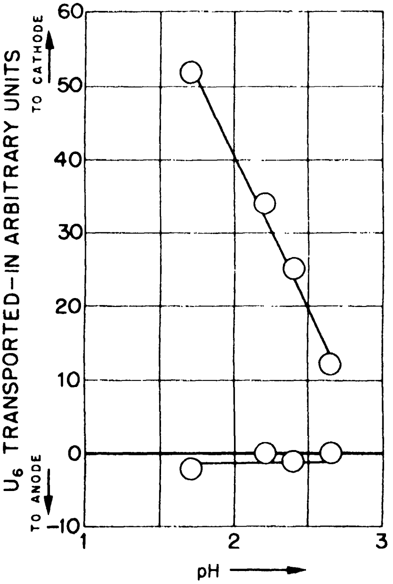


Fig. 1.7—Electrical transport of 0.004M uranyl nitrate hexahydrate in 0.05M potassium chloride and 0.05M maleate buffer; maleic acid-monosodium maleate buffer range.

In monosodium maleate-disodium maleate, U_6 forms a large amount of anionic complex from pH 5.1 to pH 6.6, corresponding to disodium maleate concentrations of 0.005M to 0.042M. The reason for the apparently greater amount of anionic complex at pH 5.1 than at pH 6.6 is not known. The formation of cation increases as the concentration of disodium maleate decreases. The situation is complicated by the fact that probably monosodium maleate as well as disodium maleate can react with UO_2^{++} to form complexes.

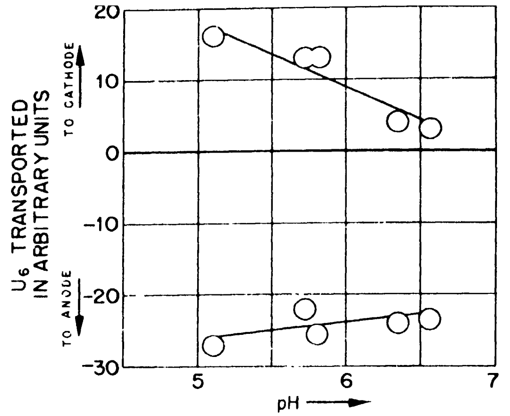


Fig. 1.8—Electrical transport of 0.004M uranyl nitrate hexahydrate in 0.05M potassium chloride and 0.05M maleate buffer; monosodium maleate-disodium maleate buffer range.

In Fig. 1.9 is shown the behavior of U_6 in 0.1M sodium glyceryl phosphate-disodium glyceryl phosphate buffer. It can be seen that in this buffer the U_6 is largely in the form of an anionic complex at all pH values investigated, and hence the glyceryl phosphate buffer is an especially good complexer for U_6. In the absence of further information it cannot be stated whether monosodium glyceryl phosphate and disodium glyceryl phosphate can react with UO_2^{++} to form complexes equally well or not. Since the anionic migration varies with pH, it might be inferred that monosodium glyceryl phosphate forms a different complex with U_6 than does disodium glyceryl phosphate, although other explanations of this behavior are also possible.

Figure 1.10 shows the behavior of U_6 in 0.1M malate buffer. Malate has been found to be an especially good complexer for U_6 from work with protein and enzymes, and the results of electrophoresis are in agreement with these other studies. Since the U_6 is mainly anionic over a wide pH range (from 2.5 to 5.5) it can be concluded that both monosodium maleate and disodium maleate are able to react with U_6 to form anionic complexes. At the lower pH values the complex is possibly a U_6-monosodium maleate, and at the higher pH values the complex may be a U_6-disodium maleate, since the anionic migration is not constant as the pH changes. However, other explanations also are possible. In the absence of further information, the results cannot be interpreted more fully.

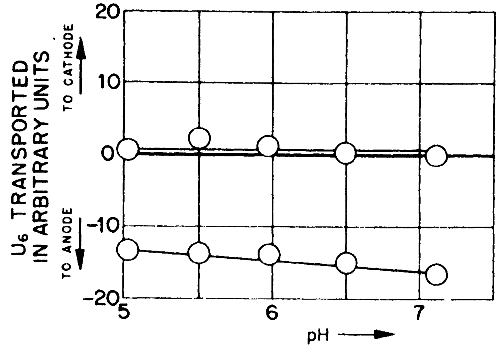


Fig. 1.9—Electrical transport of 0.004M uranyl nitrate hexahydrate with 0.05M potassium chloride in 0.1M glyceryl phosphate buffer.

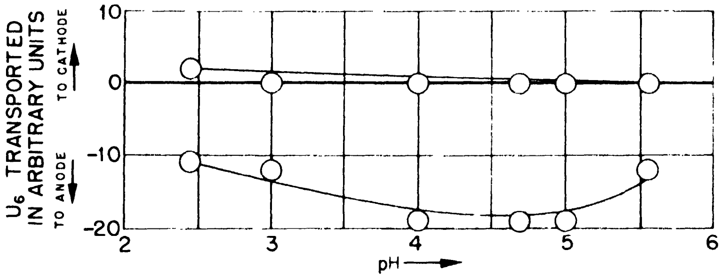


Fig. 1.10—Electrical transport of 0.004M uranyl nitrate hexahydrate with 0.05M potassium chloride in 0.1M malate buffer.

The behavior of U_6 in 0.1M citrate buffer is shown in Fig. 1.11. Citrate is an especially good complexer for U_6, and as can be seen from the curve it is able to complex the U_6 over a very wide pH range (from pH 2.3 to 5.7). It can be inferred that monosodium citrate, disodium citrate, and trisodium citrate are all able to react with U_6 to form a complex anion. From the fact that the anionic migration velocity is constant over the whole pH range investigated, it can also be inferred that one and the same complex is formed by reaction of U_6 with any of the three citrate salts.

Figure 1.12 shows the results of electrophoresis of U_6 in 0.1M carbonic acid-sodium bicarbonate buffer and in 0.1M sodium bicarbonate-sodium carbonate buffer. Both buffers are very strong complexers

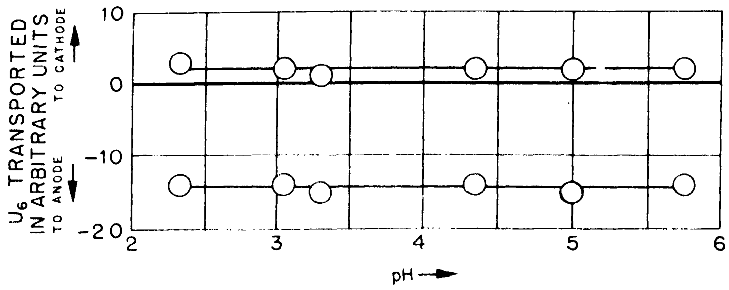


Fig. 1.11—Electrical transport of 0.004M uranyl nitrate hexahydrate with 0.05M potassium chloride in 0.1M citrate buffer.

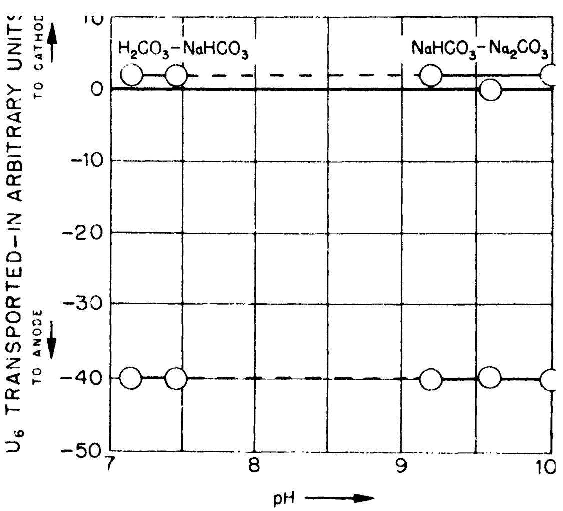
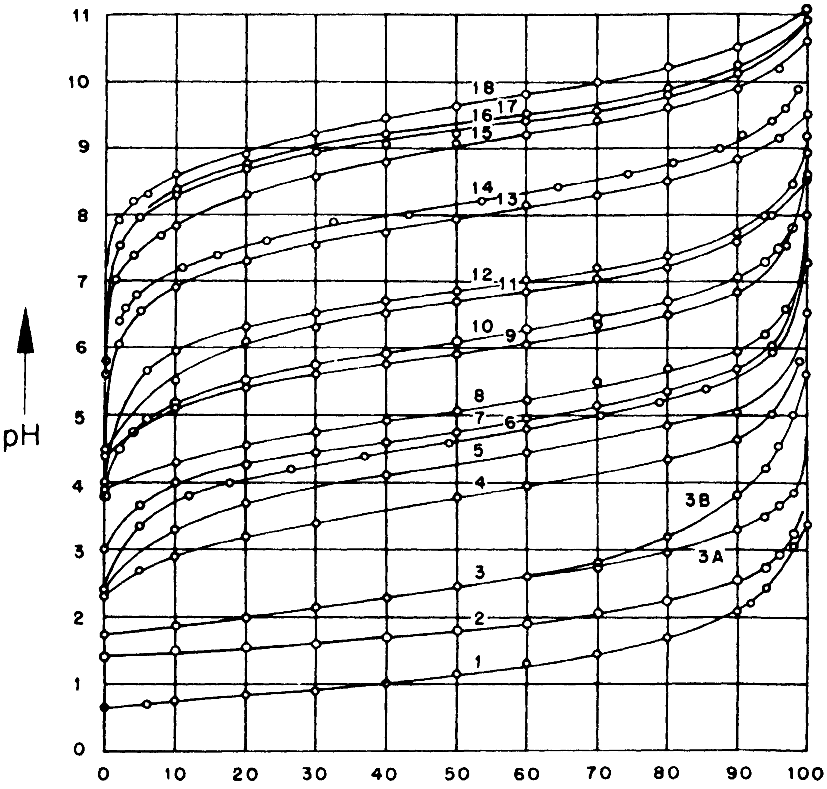


Fig. 1.12—Electrical transport of 0.004M uranyl nitrate hexahydrate in 0.05M potassium chloride and 0.1M carbonate buffer.

for U_6, since both render it anionic and completely soluble. From the fact that the migration velocities of the complex anion of U_6 are the same in the two buffers, and from independent polarographic studies, it has been concluded that the same complex of U_6 is formed in both buffer systems.

In studying the preceding figures, it is convenient to be able to tell from the pH how much of the buffer in question is in the form of ionized buffer salt, and how much of it is in the form of un-ionized buffer acid. In Figs. 1.13 and 1.14 are shown curves that give the buffer composition at a given pH. These curves correspond to 0.1M buffers,



FOR ACID-SALT BUFFERS, ABSCISSA = ML 0.1M SALT = 100 ML 0.1M ACID
FOR BASE-SALT BUFFERS, ABSCISSA = ML 0.1M BASE = 100 ML 0.1M SALT

Fig. 1.13—Buffer composition at a given pH. Curve 1, maleic acid-sodium maleate; curve 2, sodium bisulfate-sodium sulfate; curve 3, pyruvic acid-sodium pyruvate and glycine hydrochloride-glycine; curve 3a, glycine hydrochloride-glycine; curve 3b, pyruvic acid-sodium pyruvate; curve 4, lactic acid-sodium lactate and barbituric acid-sodium barbiturate; curve 5, acetic acid-ammonium acetate; curve 6, acetic acid-sodium acetate; curve 7, propionic acid-sodium propionate; curve 8, potassium biphthalate-sodium potassium phthalate; curve 9, sodium maleate-disodium maleate; curve 10, sodium glyceryl phosphate-disodium glyceryl phosphate; curve 11, potassium dihydrogen phosphate-disodium hydrogen phosphate; curve 12, sodium bisulfite-sodium sulfite; curve 13, boric acid-sodium borate in 2 per cent glucose; curve 14, veronal-sodium veronal; curve 15, boric acid-sodium borate; curve 16, ammonia-ammonium chloride; curve 17, ammonia-ammonium acetate; curve 18, glycine-sodium glycinate.

but 0.05M buffers are nearly the same. Many buffers are included which have not been employed in the work just outlined, but which may be of general use to the biochemist. These curves do not represent work of a high order of precision but are sufficiently accurate for practical studies. For example, at a given buffer composition the pH usually is accurate to ±0.1 pH unit, although in some instances the error may be greater than this. The curves were experimentally determined, using the Beckman pH meter to get the pH values.

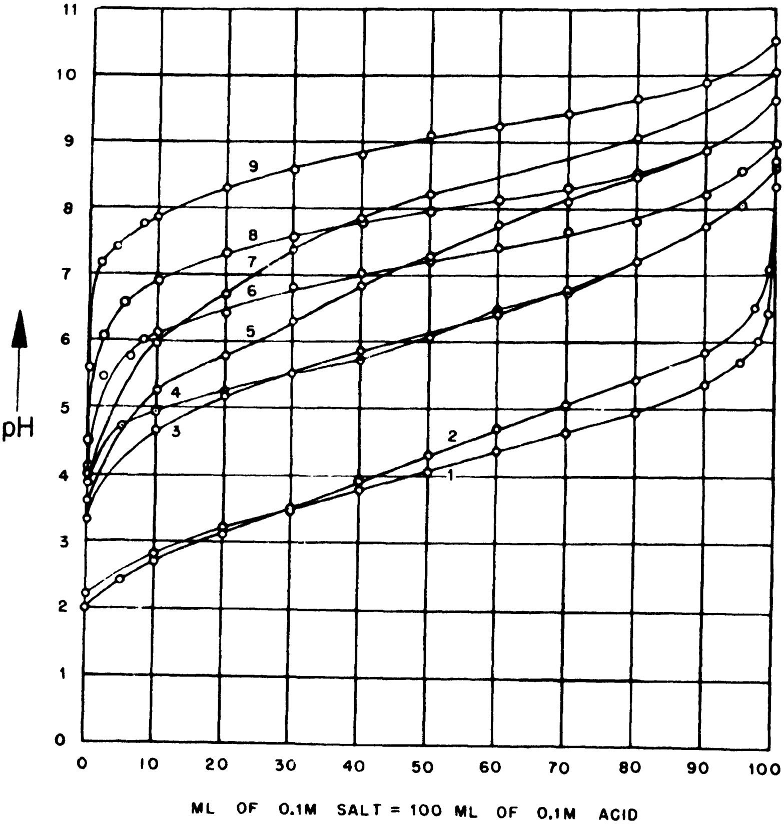


Fig. 1.14—Buffer composition at a given pH. 1, malic acid-sodium malate; 2, citric acid-sodium citrate; 3, boric acid-sodium borate in 3.3 per cent mannitol; 4, boric acid-sodium borate in 3 per cent sorbitol; 5, boric acid-sodium borate in 1.67 per cent mannitol; 6, boric acid-sodium borate in 6.6 per cent glucose; 7, boric acid-sodium borate in 0.85 per cent mannitol; 8, boric acid-sodium borate in 2 per cent glucose; 9, boric acid-sodium borate.

(b) Use of the Polarograph in Studying U_6 Complexes. The polarograph, or dropping-mercury electrode, can be used in the manner outlined by Kolthoff and Lingane in their book "Polarography"[1] to obtain information on the number of organic radicals complexing U_6 under different conditions, and to determine the dissociation constants of the various complexes formed. In this report can be found the details of a careful investigation of the acetate complexes of U_6. It was determined that either two or three acetates could complex U_6, depending upon conditions, and the dissociation constants of these complexes were determined. They will be found, together with other dissociation constants, in Table 1.5 (under oxidation-reduction studies).

It is unfortunately not always possible to obtain the detailed information about other U_6 complexes from polarographic data that were obtained for the acetate complexes. To illustrate, a study of the behavior of the polarographic waves of U_6 in bicarbonate solutions has indicated that the electrode reactions in bicarbonate are irreversible. For this reason the usual thermodynamic equations cannot be applied, and quantitative information about the number of bicarbonate (or carbonate) groups complexed with the U_6 cannot be determined, nor can the dissociation constants of the complexes be calculated.

(c) Use of the Spectrophotometer to Investigate U_6 Complexes. If the absorption spectrum of U_6 is examined under conditions where there is little free UO_2^{++} and much U_6 complex present, it is found that there is a distinct change in the amount of absorption at certain wave lengths. This is illustrated in the absorption spectra shown in Fig. 1.15. The spectra were made by plotting the absorption of uranyl acetate in 0.2M acetate buffer at pH 3.49 where there is little acetate complex and much UO_2^{++} present, and at pH 4.72 where there is much complex and little free UO_2^{++} ion. The differences in absorption shown by the two curves at 446 mμ, when absorptions at 440 mμ are made to correspond, are very noticeable. In Fig. 1.16 the difference in absorption of the two systems at 446 mμ, with absorptions at 440 mμ made to correspond, is plotted against pH. The relatively rapid transition between free UO_2^{++} cation and U_6-acetate complex as the pH is raised from 2 to 5.3 is clearly demonstrated.

(d) Use of Ferrocyanide as a Color Reagent for the UO_2^{++} Ion. The degrees of dissociation of complexes of U_6 can be compared to some extent by making use of the color reaction between UO_2^{++} and ferrocyanide. Ferrocyanide, itself an anion, reacts with the UO_2^{++} cation to give a colloidal red-colored complex, but it does not react with complex anions containing U_6. Of course the dissociation of the complex of U_6 in question is disturbed by the removal of UO_2^{++} as ferrocyanide

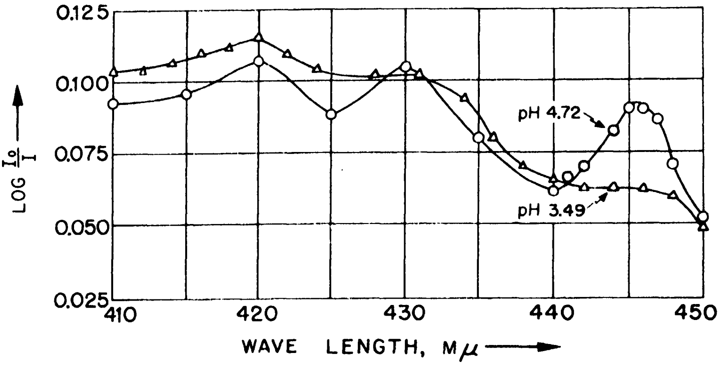


Fig. 1.15—Development of an absorption maximum at 446 mμ by a solution of 0.008M uranyl acetate in 0.2M acetate buffer as the pH is increased.

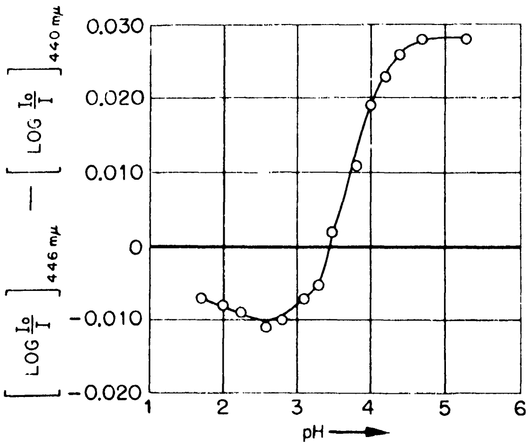


Fig. 1.16—Increase in height of absorption maximum of 0.008M uranyl acetate in 0.2M acetate as the pH is increased.

complex. For this reason the method is not well suited to show quantitative differences between highly dissociated complexes of U_6, without considerable refinement. However, it will clearly distinguish between a strong and a weak complexer for U_6. If the extent of dissociation of the ferrocyanide complex of U_6 were determined, it should be possible to calculate dissociation constants for many of the complexes of U_6. However, a complicating factor is the fact that the curve obtained by plotting color produced by the ferrocyanide test against the concentration of UO_2^{++} is nonlinear, being composed of two linear portions with a sharp inflection between them. In this work no attempt has been made to use the ferrocyanide color reaction to ascertain dissociation constants of U_6 complexes. Instead the reaction has been used simply to distinguish between weak and strong complexers of U_6.

In Fig. 1.17 are shown curves made by plotting the intensity of the ferrocyanide color caused by free UO_2^{++} ions against pH in the cases of several buffer systems. There is little doubt that the change in pH serves merely to change the ratio of uncomplexed U_6 to complexed U_6 as already explained under the topic of electrophoresis.

It might be questioned whether change in pH would also change the amount of $Fe(CN)_6^{==}$ ion or alter the reaction between this ion and the free UO_2^{++} ion. In order to show (indirectly) that pH changes probably do not interfere in either of the two latter ways, the color produced by Cu^{++} in the presence of ferrocyanide was plotted against pH, using acetate buffer. Cupric acetate is sufficiently dissociated even at pH values as high as 7 or thereabouts so that a strong color reaction is obtained, which is only slightly less intense than the color produced in acid solutions. But when citrate was added, which strongly complexes Cu^{++} at pH values in the neighborhood of 6 or 7.0, the ferrocyanide color reaction is completely inhibited. The apparent increase in color reaction at the pH range of 4 to 5.4 is an artifact caused by

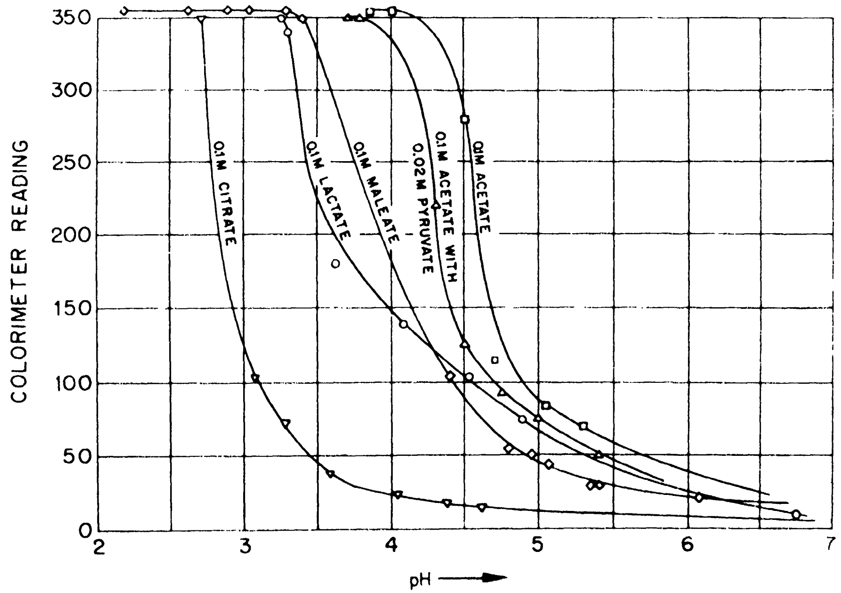


Fig. 1.17—Hexavalent uranium ferrocyanide color with various buffer anions. All solutions contain 0.0002M uranyl nitrate, 2 per cent $K_4Fe(CN)_6$, and 0.2 per cent Na_2SO_3.

the appearance of a precipitate in the solution at this pH range, which was not centrifuged off.

The results of the experiments just described concerning the cupric ferrocyanide color reaction in acetate and citrate buffers are shown in Fig. 1.18.

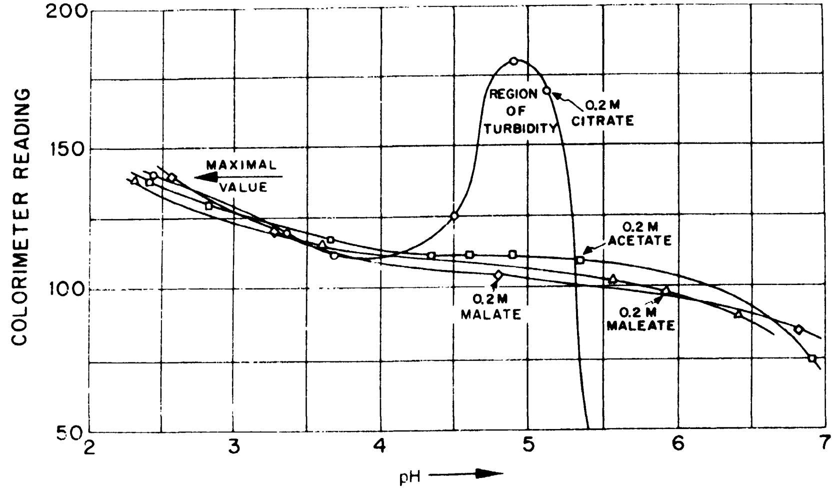


Fig. 1.18—Effect of pH and buffer ions on the formation of the cupric ferrocyanide color complex. All solutions contain 0.004M $CuSO_4$, 2 per cent $K_4Fe(CN)_6$, and 0.2 per cent Na_2SO_3.

Returning to Fig. 1.17, it can be seen that an idea can be gained of the relative dissociation of the complexes of U_6 with the various buffer anions employed at a given pH value by marking off the intercepts of a vertical line at the given pH with the curves corresponding to the buffers in question. The higher the intercept, the more free UO_2^{++} is produced by dissociation of the complex, and hence the greater the dissociation of the complex in question. It must be kept in mind, however, as already mentioned, that distortion is produced by the interference of the ferrocyanide reaction with the dissociation equilibrium of the U_6-buffer anion complex.

(e) Use of Titration Experiments to Investigate the Complexing of U_6 with Proteins and Amino Acids. In Fig. 1.19 are shown various titration curves obtained by titrating crystallized hen's egg albumin with acid and base in the presence and absence of uranyl nitrate. The amount of acid or base added is shown on the horizontal axis, and the ordinate is pH. The titration curve for uranyl nitrate also is included. In detail the curves were obtained as follows:

Curve A was made by titrating 5 ml of 6 per cent solution of once-recrystallized, dialyzed egg albumin with 0.1N HCl and 0.1N NaOH. The protein was close to the isoelectric point initially as can be seen from the point of intersection of the titration curve with a vertical line drawn through the zero point on the horizontal axis.

Curve B was obtained by plotting the results of a titration of 1 ml of 0.05M uranyl nitrate with acid and base. The original pH of the uranyl nitrate solution was 3.6.

Curve C was made by titrating a mixture of 5 ml of 6 per cent egg albumin and 1 ml of 0.05M uranyl nitrate. The starting pH was 3.7.

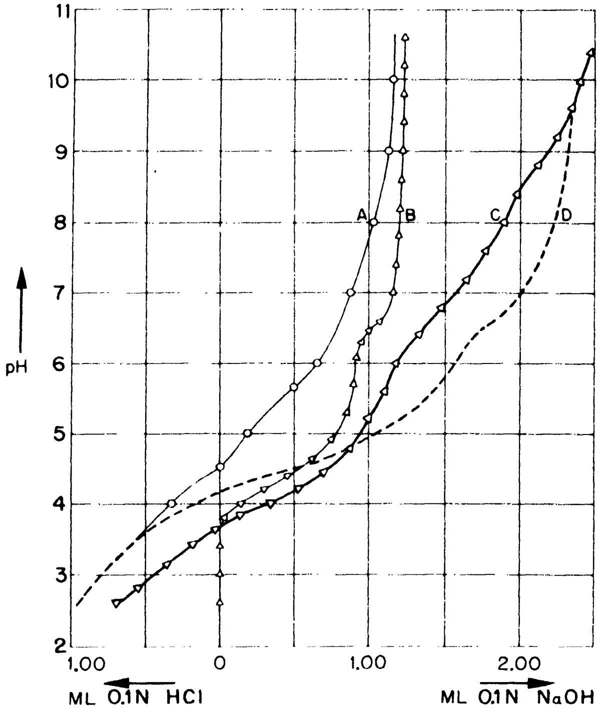


Fig. 1.19—Effect of U_6 on the titration of egg albumin. Curve A, 5 ml of a 6 per cent solution of once-recrystallized, dialyzed egg albumin; curve B, 1 ml of 0.05M uranyl acetate; curve C, 5 ml of 6 per cent egg albumin plus 0.05M uranyl nitrate hexahydrate; and curve D, A plus B (calculated).

The values for curve D were calculated by adding the values on curve A and curve B.

All the titration data determined experimentally (i.e., data corresponding to A, B, and C) were corrected before being plotted by subtracting the titration curve of distilled water from them. The curve was made by titrating distilled water with acid and base. Since all the solutions to be titrated were made up to an initial volume of about 20 ml with distilled water, this volume of distilled water alone was used in determining the distilled-water titration curve. For the sake of simplifying the diagram, the distilled-water titration curve is not shown, and all three experimentally determined titration curves were plotted after the distilled-water curve had been subtracted from each of them.

By comparing the calculated curve D with the experimental curve C, it can be seen that the uranyl nitrate and the albumin interact in such a manner as to result in reducing over considerable pH ranges both the acid- and the base-combining powers of the system below the values calculated on the assumption of no interaction. It will be noted that the crossing point of the two curves occurs very close to the isoelectric point of the albumin. There is no change in acid- or base-combining power of the system at this crossing point, perhaps partly because the albumin has feeble acid- or base-binding properties at its isoelectric point, which is close to the crossing point. It must not be concluded, however, that there is no chemical interaction between the uranyl nitrate and albumin at or near the isoelectric point of the albumin, since egg albumin can be precipitated by uranyl nitrate at or near the isoelectric point, and the washed precipitate contains U_6.

It is most probable that the decrease in acid-combining power of the U_3-albumin system over a large pH range below the isoelectric point is to be ascribed to a considerable degree to the formation of U_6-carboxyl complexes. It must be kept in mind that acid groups of proteins are titrated in a negative sense by acid on the portion of the titration curve below the isoelectric point. Formation of uranyl carboxyl complexes apparently decreases the ionization of the carboxyl groups. Liberation of HNO_3 by enhanced hydrolysis of the uranyl nitrate in the presence of protein also may contribute to the lowering of acid-combining power of the U_6-protein system. On the other hand, it is not thought that U_6 reacts with basic groups of proteins, and hence the most likely explanation for the reduction of base-combining power of the U_6-albumin system above the isoelectric point is that the interaction between ionized acid groups of the protein and U_6 lowers the base-combining power of the U_6 but has little or nothing to do with the base-combining power of the protein.

Similar titration curves were obtained from a study of the interaction of uranyl nitrate with crystallized horse hemoglobin and crystallized beef hemoglobin. It was found that the crossing points of the curves corresponding to C and D in the egg-albumin experiment were at somewhat lower pH values than the isoelectric point of hemoglobin but still were reasonably close to it. It was also found that the crossing point was independent of the concentration of uranyl nitrate over concentration ranges practical to employ.

In Fig. 1.20 curves obtained from titration experiments with crystallized beef hemoglobin are shown. It will be noted that the crossing point is the same for two different concentrations of uranyl nitrate.

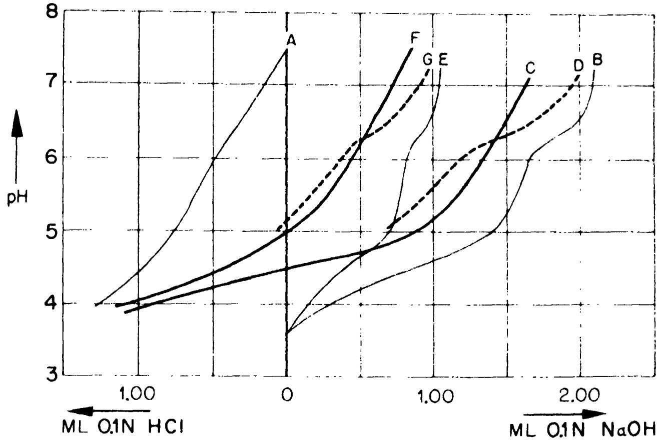


Fig. 1.20—Effect of U_6 on the titration curve of beef hemoglobin. Curve A, 5 ml of 5 per cent beef hemoglobin; curve B, 1 ml of 0.1M uranyl nitrate hexahydrate; curve C, 1 ml of 0.1M uranyl nitrate hexahydrate plus 5 ml of 5 per cent beef hemoglobin; curve D, A plus B (calculated); curve E, 0.5 ml of 0.1M uranyl nitrate hexahydrate (calculated from B); curve F, 0.5 ml of 0.1M uranyl nitrate hexahydrate plus 5 ml of 5 per cent beef hemoglobin; curve G, A plus E (calculated).

It is highly probable that U_6 which combines with protein below the isoelectric point must be forming complexes with still-ionized carboxyl groups. Sufficient lowering of the pH to repress completely the ionization of the carboxyl groups in the protein will prevent complexing of U_6 with the protein. At low pH values the only type of combination of U_6 with protein that appears possible is simple salt formation between an already present anionic complex of U_6 and ionized basic groups of the protein. Such combination would of course be very labile and would not change the acid-combining power of the system. It should be kept clearly in mind in considering all the titration experiments that it is the acid- or base-combining power of the entire system which is measured in titrating mixtures of protein and uranyl nitrate, not the acid- or base-combining power of either component separately.

In Fig. 1.21 is shown the result of titrating the amino acid serine in the same way as described for the titration of protein in the presence of U_6. It can be seen that there is far less change in the acid- and base-combining power caused by U_6 than in the case of proteins. For some reason there appears to be less interaction between amino acids and U_6 than between proteins and U_6. The amino acid glycine yielded essentially the same results as serine.

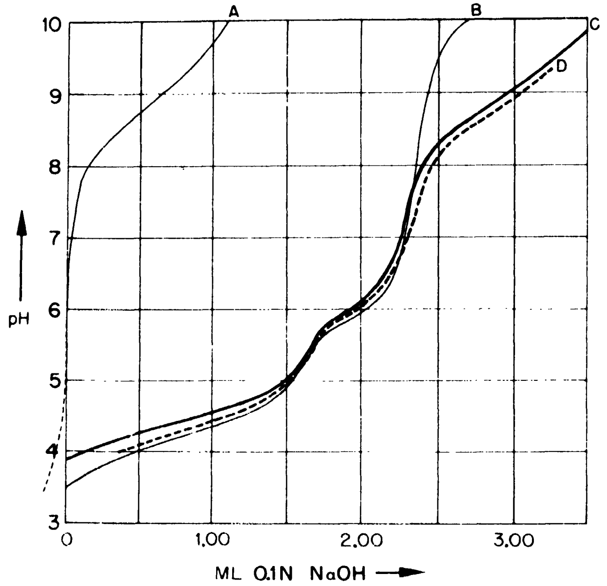


Fig. 1.21—Effect of U_6 on the titration curve of serine. Curve A, 1 ml of 0.1M serine; curve B, 1 ml of 0.1M uranyl nitrate hexahydrate; curve C, 1 ml of 0.1M serine plus 1 ml of 0.1M uranyl nitrate hexahydrate; curve D, A plus B (calculated).

The reader is referred to Chap. 13 for a more detailed presentation of the experimental work dealing with the titration of proteins in the presence of uranyl nitrate.

(f) Use of the Ability of a Given Complexer to Dissolve, at pH 5 to 6, Protein That Has Been Precipitated by U_6. It has been found that the relative efficiency of anions as complexers of U_6 can be compared roughly by testing their ability to dissolve a protein such as egg albumin that has been precipitated by uranyl acetate at a pH range of 5 to 6. When sufficient of the complexer has been added, the U_6 will be removed from the precipitated protein because of the formation of anionic complex with the added complexing agent. The anionic complexes of U_6 do not have any ability to precipitate protein, at least above the isoelectric point of the latter. If the pH is raised to 7.0 or higher without the addition of complexer, the protein-U_6 precipitate will tend to dissolve, since the acid groups of the protein become sufficiently good complexers to keep both the protein and the U_6 in solution. Hence, in the testing of complexers by their ability to dissolve protein precipitated by U_6, the pH must be properly controlled.

A somewhat less satisfactory method for testing the ability of materials to complex U_6 is to test their action in dissolving a freshly precipitated oxide of U_6 made by adding alkali to uranyl nitrate. In this case, contrary to the case in which precipitated protein is employed, the solution of the precipitate caused by addition of the complexer tends to be sluggish.

(g) Discussion of Results Obtained in Comparing Various Complexers of U_6. In regard to the results obtained from studying the complexing action of various materials on U_6 by the methods listed previously, it can be stated briefly that bicarbonate, citrate, and malate form with U_6 slightly dissociated complexes which are strongly anionic in character, and that acetate and lactate form complexes which dissociate to an easily detectable amount, depending upon the concentration of the complexing agent. Phosphate apparently can form either a precipitate with the UO_2^{++} cation or a complex anion with U_6, depending upon the pH of the solution. Organic monophosphate esters such as glyceryl phosphate and glucose-1-phosphate appear to be good complexers for U_6. Protein can form a precipitate by reacting with the UO_2^{++} cation. For precipitation to occur, however, inorganic salt must be present in many cases. Egg albumin and serum globulin do not require inorganic salt for precipitation with U_6. The precipitation of protein by U_6 usually is best at a pH range in the neighborhood of 4.5 to 5.0. As has already been mentioned, protein can form soluble complexes with U_6 at pH 7.0 or higher.

For a rough comparison of the complexing affinities of various anions for U_6, reference is made to Table 1.3.

Table 1.3 — Relative Affinities of Complexing Anions for U_6

Group I	Group II	Group III
Acetate	Pyruvate	Malate
Lactate	Glyceryl phosphate	Citrate
Maleate	Proteinate	Bicarbonate
Phosphate		Carbonate

The complexing affinity for substances in Group III is greater than that for substances in Group II, and the complexing affinity of substances in Group II is greater than that of materials in Group I. The relative positions of the anions within a given group are not known with certainty. The distinction between Groups I and III is very sharp, but the distinctions between Groups I and II and between Groups II and III are less sharp.

It should be stated in the concluding remarks about simpler complexers of U_6 that SH groups in compounds such as cysteine and glutathione do not appear to have any complexing affinity for U_6.

(h) Complexers of U_6 of Physiological Importance. In considering the chemistry of U_6 in the body, the problem of its distribution between protein and bicarbonate in blood plasma was found to be of great importance, since protein and bicarbonate are both strong complexers for U_6 and both are present in plasma in far higher concentrations than are other possible U_6 complexers. This distribution problem has been attacked in two ways — indirectly and directly.

The indirect attack was to compare the electrophoretic anionic mobilities of U_6 in the presence of 1 per cent egg albumin alone, in the presence of 0.025M bicarbonate alone, and in the presence of 1 per cent egg albumin plus 0.025M bicarbonate (figures rounded off). The results of this experiment are shown in Fig. 1.22. Since the anionic mobility of the U_6 in bicarbonate plus egg albumin is almost as great as that in bicarbonate alone and much greater than in egg albumin alone, it can be seen that the albumin cannot compete successfully with the bicarbonate in complexing the U_6. The major portion of the U_6 becomes bicarbonate complex.

The direct attack was to study the ultrafilterability of U_6 in beef serum in the presence of varying amounts of bicarbonate. Relatively coarse collodion ultrafilters were used which were, however, not permeable to plasma protein. The results of this work are shown in Fig. 1.23, where the bicarbonate is measured as volumes per cent of CO_2, which is a sufficiently accurate means of measurement for this type of experiment. The filterable U_6 varies as an increasing function of the bicarbonate concentration in an S-shaped curve and becomes nearly zero in the absence of bicarbonate. It can safely be assumed that most of the filterable U_6 exists as bicarbonate complex. Since protein itself is nonfilterable, since sufficient bicarbonate is present to render the U_6 100 per cent filterable in the absence of protein, and since it is known that U_6 has a high affinity for protein, it follows that the nonfilterable U_6 is principally or entirely present as a U_6-protein complex.

In animals with normal CO_2-combining capacity, the U_6 should be nearly equally divided as bicarbonate complex and protein complex. Traces of other U_6 complexes also must be present. If an animal were to be flooded with bicarbonate by continuous infusion of bicarbonate, a large fraction of injected U_6 should be rapidly excreted through the kidneys as bicarbonate complex, since the filterability of blood U_6 would be high and since the urine itself would be high in bicarbonate. In Chap. 15 on the mechanism of action of uranium compounds on the body, it is demonstrated that this result can be obtained experimentally.

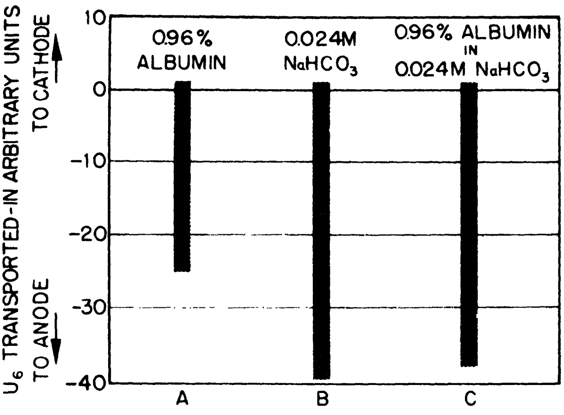


Fig. 1.22—Electrical transport of 0.004M uranyl nitrate hexahydrate at pH 7.3 in the presence of 0.96 per cent egg albumin (A), 0.024M sodium bicarbonate (B), and 0.96 per cent egg albumin in 0.024M sodium bicarbonate (C).

In the foregoing paragraphs, serum protein has been considered in toto as a complexer of U_6. In order to investigate the distribution of U_6 among the various known plasma protein components, an electrophoresis of plasma (which originally had been oxalated) in the presence of U_6 was carried out by Alling. It was concluded that some U_6 is attached to albumin, various globulin fractions, and fibrinogen, because the electrophoretic mobilities of all these fractions were increased slightly by the presence of small amounts of U_6. These changes in mobilities were not due to protein damage, since the original mobilities were observed after exhaustive dialysis of the uranyl acetate-plasma mixture at pH 8.5 in the presence of sodium malate, a strong complexer for U_6. This last statement is in agreement with findings in enzyme and protein studies that U_6 in general does not denature or damage proteins, provided that the pH and other conditions are kept within limits ordinarily tolerated by the protein in question.

Hexavalent uranium can complex firmly with denatured as well as with the native protein. This fact furnishes the basis for a quantitative separation of U_6 from substances interfering in the fluorometric determination of U_6. The U_6 is allowed to complex with egg albumin in dilute acetate buffer, the pH is adjusted to about 4.8, and the albumin then is heat-denatured. The denatured albumin with which the U_6 remains combined is centrifuged down, leaving behind most of the interfering substances, such as, for example, the calcium and most of the phosphate of bone ash. See Chap. 2 for the details of this method, which was developed by W. F. Neuman's group.

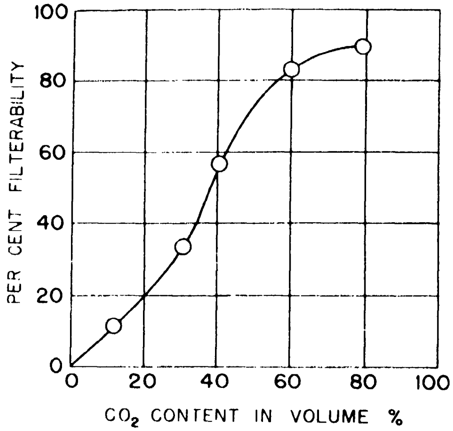


Fig. 1.23—Filterability of U_6 in beef serum. Concentration of U_6, 5.7 μg of uranium (metal) per cubic centimeter.

2.2 Chemistry of U_4.* Less work has been done on the chemistry of U_4 than on the chemistry of U_6, partly because of technical difficulties but chiefly because it appears that U_4 is not produced in appreciable quantities in the body by reduction of U_6 and apparently cannot enter the body in significant amounts unless directly injected.

Many of the methods for investigating complexes of U_6 that have been described cannot be applied to an investigation of complexes of U_4. For instance, the important method of electrophoresis is difficult to apply to U_4 complexes because of the low solubility of U_4 in solutions that are not strongly acidic. The polarographic method can, however, be applied to an investigation of U_4 chemistry, as can the simple method of testing complexers of U_4 by noting their ability to dissolve protein such as egg albumin that has been precipitated by U_4. Some information might also be gained by use of the spectrophotometer.

By testing the ability of a given complexer to dissolve protein precipitated by U_4, it has been found that citrate and lactate are good complexers for U_4. Acetate is known from polarographic studies to be a complexer for U_4, but it does not appear to be so good a complexer as citrate. In carrying out experiments of this sort, it is convenient to precipitate the egg albumin with UCl_4 that has been neutralized to pH 5.0 with sodium acetate. If UCl_4 is used to precipitate the egg albumin without previous partial neutralization, the suspension of precipitate must quickly be adjusted to pH 5.0 with 0.1N NaOH, or the low pH will cause the albumin to become denatured so that it will not dissolve when the complexer is added.

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*U_4 will be used as a general term for tetravalent uranium, including the UO^{++} cation and complexes of U_4, which presumably are anions.

Bicarbonate also can complex U_4. This has been established by W. F. Neuman's group who found that 0.1M bicarbonate would remove U_4 quantitatively from denatured egg albumin. However, it was also found that 0.025M bicarbonate would not remove U_4 from denatured egg albumin, whereas it would remove a large amount of U_6 from denatured egg albumin. Therefore, it can be inferred either that bicarbonate is a better complexer for U_6 than for U_4, or that protein is a better complexer for U_4 than for U_6, or that both of these statements are true.

Protein is apparently able to form soluble complexes with U_4, since egg albumin that has been precipitated by U_4 as described above can be made to redissolve by increasing the pH of the solution to 7.0 or above. Provided that the pH is kept below 8.0, it appears likely that this effect occurs mainly as the result of soluble U_4-protein-complex formation and not as a result of splitting off the U_4 from the protein to yield an insoluble oxide. In this respect U_4 behaves similarly to U_6; however, on prolonged standing at pH 7 to 8 it appears quite possible that the U_4 may gradually pass over to the form of an insoluble oxide which may remain colloidally suspended or which may gradually precipitate from the solution. A careful investigation of this point would be well worth while.

Provided that the pH and other conditions are kept within limits ordinarily tolerated by a given protein, precipitation of the protein by U_4 has little or no tendency to cause denaturation (see Chap. 13).

One sharply defined chemical difference between U_6 and U_4 is important from the biochemical standpoint. This difference is that U_6 precipitated from uranyl nitrate or acetate by the addition of dilute NaOH can be made to redissolve by the addition of strong complexers like citrate or bicarbonate, whereas this behavior does not hold for U_4. Therefore, if any small amount of oxide of U_6 should be formed locally on injection of U_6 into an animal, it would rapidly be brought into solution as the result of formation of the soluble bicarbonate complex. But in the case of U_4 it is quite certain that any locally precipitated U_4 oxide would remain insoluble, and even a colloidal oxide probably would persist as such in plasma. To illustrate this behavior of U_4, the following test-tube experiments are cited: Rapid addition of dilute UCl_4 solution, with vigorous stirring, to an excess of 0.1M bicarbonate solution results in a perfectly clear solution, indicating the formation of a soluble bicarbonate complex of U_4. However, slow addition of the UCl_4 solution to the bicarbonate without vigorous stirring yields some insoluble precipitate, presumably U_4 oxide, which will not dissolve in excess bicarbonate.

From a consideration of these results it appears likely that injection of U_4 into an animal might give rise to the formation of a certain amount of U_4 oxide as well as U_4 proteinate in the plasma, and that the ratio of these two substances might vary with the mode of injection of the U_4. However, it also appears likely that a colloidal U_4 oxide would behave in the body in many respects similarly to a U_4 proteinate, in being unable to pass readily through cell walls or through the glomerulus and in tending to accumulate in the reticulo-endothelial system. It is unfortunate that owing to lack of time this whole matter could not have been investigated carefully. It might be possible to distinguish U_4 oxide from U_4 proteinate in plasma by the ultracentrifuge technique.

Ultrafiltration studies carried out on U_4 in serum have indicated that U_4 is only slightly ultrafilterable from serum, and that an increase in the bicarbonate concentration of the serum produces only a very small effect in enhancing the ultrafilterability of the U_4, as shown in Fig. 1.24. This result was to be expected from the qualitative chemical findings already presented.

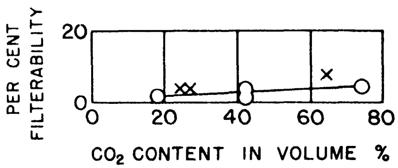

Fig. 1.24—Filterability of tetravalent uranium in rabbit serum. x, 2 μg of uranium (metal) per milliliter; ○, 60 μg of uranium (metal) per milliliter.

Finally, it was determined in experiments with animals (see Chap. 15) that relatively little uranium appeared in the urine after intravenous injection of U_4, that the U_4 had less tendency to be concentrated in the kidneys than did U_6, and that infusion of the animals with bicarbonate produced very little effect in increasing the urinary excretion of uranium. These results are to be expected from the previously presented chemical information.

2.3 Oxidation-Reduction Studies. The chief reason for studying the oxidation-reduction properties of systems containing uranium in different valence states was, of course, to attempt to predict the final state of uranium, with respect to valence, after its entry into the animal body. The oxidation-reduction problem, which arises when a study is made of uranium compounds in biological fluids, presents technical difficulties that are extremely difficult to surmount directly. Much of the information applicable to biological systems that has been gained about the behavior of uranium compounds, with respect to oxidation and reduction, has been gained by extrapolations or by inference from indirect experiments. The literature was of little value because all the previous studies appear to have been conducted on uranium compounds in strongly acid solutions. In spite of the above-mentioned difficulties, the results obtained from in vitro studies agree reasonably well with results obtained from in vivo studies.

In attacking the oxidation-reduction problem the following methods have been employed:

1. Oxidation-reduction reactions of U_6 or U_4 were studied in the presence of such inorganic reagents as sodium hydrosulfite and hydrogen peroxide, as well as a number of biological materials including ascorbic acid, hemoglobin and oxyhemoglobin, cytochrome C (oxidized and reduced), and lactic acid in the presence of codehydrogenase I plus lactic dehydrogenase.
2. The oxidation of U_4 by molecular oxygen was studied.
3. Indicator-dye studies using methylene blue were carried out.
4. Smooth-platinum-electrode studies were made.
5. Polarographic studies were carried out.

Before giving detailed results, it should be stated first, that the U_6,U_4 system is rather sluggish from the standpoint of oxidation-reduction reactions; second, that U_5, the pentavalent form of uranium, is quite unstable in solution and rapidly undergoes dismutation to yield U_6 plus U_4; and third, that U_3 is very unstable in solution and reacts rapidly with molecular oxygen to yield U_4 as the immediate oxidation product. The U_4,U_3 system has such a negative oxidation potential and U_3 undergoes such rapid oxidation by molecular oxygen that it appears out of the question that U_3 could be stable in the body, and therefore this valence state of uranium will not be considered further.

(a) Chemistry of U_5. The chemistry of U_5 is most conveniently considered under the general heading of oxidation-reduction reactions of uranium, since it is not stable and can be demonstrated in solution only as an intermediate in the oxidation of U_4 or the reduction of U_6.

U_5 could not exist in the body except in trace amounts compared to the amounts of U_6 or U_4 present, owing to its instability, which is caused by a reaction of dismutation. It would be convenient to consider U_5, the oxidation-reduction intermediate between U_6 and U_4, as analogous to the free radicals that exist in systems of organic dye materials. However, the dismutation of U_5, which yields U_4 plus U_6, is a relatively sluggish process, while the dismutation of organic free radicals is extremely rapid. For this reason the equations of Michaelis[2] cannot be applied without modification to U_5, since they are based upon the assumption of an extremely rapid dismutation reaction.

Although the kinetics of dismutation of U_5 are sluggish relative to the dismutation of organic free radicals, the dismutation process is sufficiently rapid, and so little U_5 is present at equilibrium that it is somewhat difficult to demonstrate directly the presence of U_5 in solution. If UCl_5 is added to water, a greenish solution is at once produced, which by spectrophotometric analysis can be demonstrated to consist of equimolar portions of U_6 and U_4. This is illustrated in Fig. 1.25. The absorption curve obtained by spectrophotometric study of a solution of UCl_5 can be very nearly superimposed upon a curve made by adding the absorption curves obtained from U_6 and U_4 in acid solution, each at half the total uranium concentration present in the solution made by dissolving UCl_5. The forms of the observed and calculated curves are practically identical. The differences occurring in magnitude of absorption at the left-hand portions of the curves are most probably caused by a slight oxidation of U_4 to U_6 by atmospheric oxygen in the solution made by dissolving the UCl_5. In order to keep the U_4 entirely in the reduced state, a small amount of hydrosulfite was added, but this could not be done in the case of the solution made by dissolving UCl_5, since hydrosulfite reduces U_6.

The only other possible interpretation of the spectroscopic results just outlined would be that solid UCl_5 itself is really an equimolar mixture of UCl_6 and UCl_4. As far as can be determined, the latter possibility does not correspond to the facts.

Although the kinetics of U_5 dismutation are sluggish compared to the kinetics of the dismutation of organic free radicals, U_5 is very unstable in aqueous solutions as has already been stated and demonstrated. The dismutation reaction must involve hydrogen or hydroxyl ion. In acid solution the reaction can be written as follows:

$$UO_2^{++} + UO^{++} + H_2O \rightleftharpoons 2UO_2^{+} + 2H^{+}$$

The constant for this reaction as written has been calculated from polarographic data and is 25×10^{-10} at approximately 25°C.

Before leaving the chemistry of U_5, it should be stated that this material is probably not of importance, as such, in producing the biological effects of uranium poisoning, as can be seen from the foregoing material. But UCl_5 can produce very intense damaging effects locally because of its hydrolysis to yield free hydrochloric acid. The following equation for the dismutation of UCl_5 includes this hydrolysis:

$$2UCl_5 + 3H_2O \rightleftharpoons UO_2Cl_2 + UOCl_2 + 6HCl$$

It must be remembered that UO_2Cl_2 and especially $UOCl_2$ are in

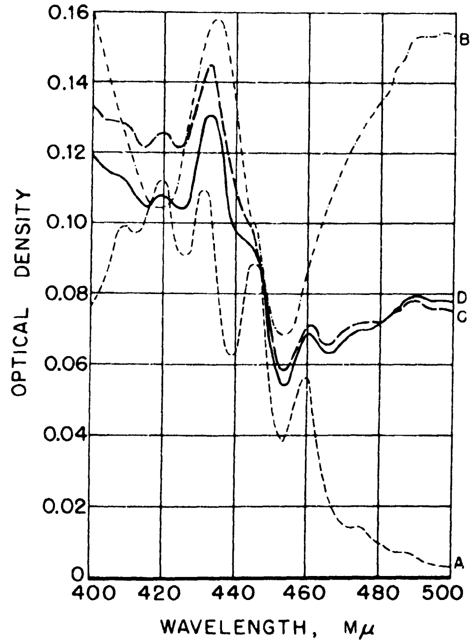

Fig. 1.25—Spectrophotometric investigation of dismutation of UCl_5; 0.008M concentration of uranium in 0.2M acetate buffer at pH 4.8. Curve A, U_6; curve B, U_4; curve C, U_5; curve D, average of A and B.

themselves acidic materials. So much free HCl is produced when a particle of UCl_5 gets into the eye, for instance, that extensive local damage is bound to occur (see Chap. 9).

(b) Reduction of U_6 by Chemical Methods. U_6 is readily reduced by sodium hydrosulfite to U_4 in solutions of any pH from 1 to 8. If the pH is not above 5.0, a transitory red color appears which quickly changes into the green color of U_4; $Na_2S_2O_4$ does not reduce U_4, nor does it form a colored complex with U_4. This transitory red-colored intermediate is undoubtedly U_5. It also can be observed with somewhat more difficulty by oxidizing U_4 with hydrogen peroxide. Both platinum-electrode studies and polarographic studies have indicated that the kinetics of U_5 dismutation are slow enough to permit momentary observation of U_5 in solution under the proper conditions.

Sodium formaldehyde sulfoxylate, sodium sulfite, sodium bisulfite, and hydrogen sulfide do not reduce U_6. If ascorbic acid is added to uranyl acetate at pH 5 to 5.5, a red color appears, as is well known. The same phenomenon is observed when dioxymaleic acid is substituted for ascorbic acid. The red color persists indefinitely and is probably caused by red-colored complexes of U_6 with ascorbic acid and dioxymaleic acid rather than by reduction of U_6 to U_5.

It has not been possible to cause reduction of U_6 by the lactate-pyruvate system in the presence of crystalline lactic dehydrogenase and codehydrogenase I, at pH 6 to 7. (It is necessary to add lactic acid dehydrogenase and coenzyme I in order to establish equilibrium between the lactate and the pyruvate at the indicator electrode.) The lactate-pyruvate system constitutes an oxidation-reduction system with one of the most negative potentials known to occur in living cells of animal tissue. The E_0' for this system is about -0.26 volt based on hydrogen, using the sign convention ordinarily employed by biochemists in which the oxygen potential is positive. The term E_0' is defined as the experimentally observed potential of a system at a given pH when the activities of the oxidized and reduced forms of the system are equal. Since the hydrogen-ion activity is not considered equal to 1 as in the case of E_0 (standard potential value), it is seen that E_0' varies with pH if the oxidation-reduction potential of the system is pH dependent. In biological systems the observed potential generally is pH dependent, and the E_0' values of various biological systems are usually compared at pH 7.0.

A comparison of the E_0' values of biological systems gives one a comparison of the relative oxidizing powers of these systems. A high positive oxidation potential (E_0') indicates that, relatively speaking, the system is a strong oxidant, and a low negative potential indicates that, relatively speaking, the system is a strong reductant. However, it is not uncommon to find that no reaction takes place between two biological systems of different E_0' values simply because no mechanism exists to make the reaction possible. Differences in E_0' values therefore do not indicate that two systems will react, but they predict the outcome of any reaction that does occur.

(c) Oxidation of U_4 by Chemical Means. As has already been stated, U_4 in solution can be oxidized by hydrogen peroxide, yielding a transitory red-colored intermediate that is difficult to observe because of its very short life. The red color is instantly changed to yellow, and as this happens a precipitate of U_6 peroxide appears. Of course U_4 can be oxidized to U_6 by many inorganic oxidizing agents, such as persulfate and dichromate. It has not been possible to observe the red-colored intermediate with agents other than peroxide, however.

If oxyhemoglobin and U_4 are mixed at pH 5.0 to 5.5 in the presence of acetate buffer, the oxyhemoglobin is slowly deoxygenated to yield hemoglobin, and the U_4 presumably is oxidized to yield U_6.

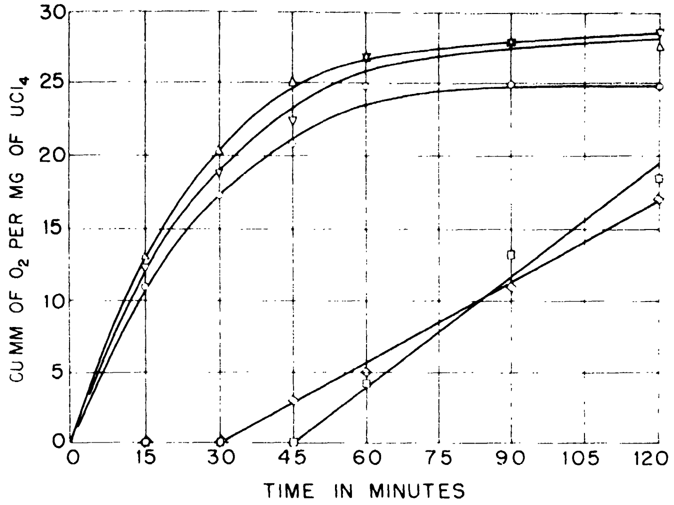

Fig. 1.26—Effect of acetate buffer on the oxidation of UCl_4 by molecular oxygen. △, 6.9 mg of UCl_4 in water; ▽, 4.0 mg of UCl_4 in water; ○, 6.0 mg of UCl_4 in water; □, 9.9 mg of UCl_4 in 0.1M acetate buffer, pH 5.0; ◇, 5.0 mg of UCl_4 in 0.1M acetate buffer, pH 5.0.

At pH values up to 6.0 and undoubtedly higher, U_4 is readily oxidized to U_6 by molecular oxygen. At the higher pH values a complexer such as acetate buffer must be added to prevent precipitation of the U_4. The presence of buffer changes the kinetics of the oxidation from those observed when UCl_4 in water without buffer is studied, as is shown in Fig. 1.26. The rate of oxygen uptake in this and following experiments was measured by use of the Warburg machine. The gaseous phase in the Warburg vessels was air. One-tenth molar acetate buffer of the desired pH was placed in the main compartment of the vessels, when buffer was used, and UCl_4 was weighed into the side arms of the vessels by difference, in amounts varying from 3.7 to 7.0 mg. The oxygen uptake in cubic millimeters per milligram of UCl_4 under standard conditions is plotted against time in minutes.

The oxidation of U_4 by molecular oxygen at pH 5 in 0.1M acetate buffer is retarded to a marked degree by cysteine, and to a somewhat lesser degree by glutathione. Ascorbic acid alone has little effect in retarding the oxidation of U_4 by molecular oxygen, but ascorbic acid plus cysteine together are more effective than cysteine alone in retarding the oxidation.

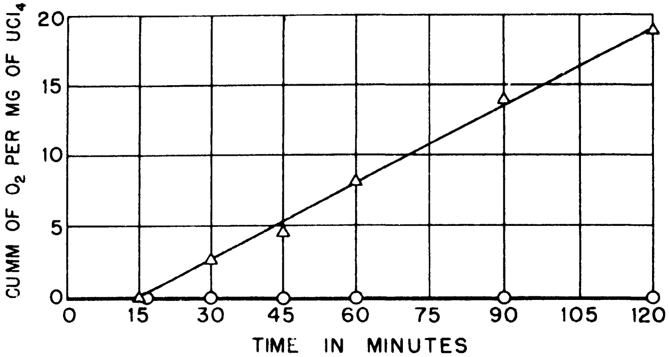

Fig. 1.27—Oxidation of UCl_4 by atmospheric oxygen, alone and in the presence of cysteine; pH 5.0 with 0.1M acetate buffer. Molar ratio of cysteine to UCl_4 equals 1.2 : 1. △, UCl_4 alone; ○, UCl_4 and cysteine.

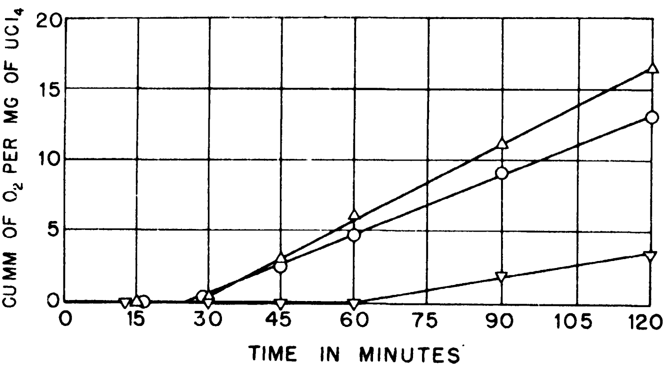

Fig. 1.28—Oxidation of UCl_4 by atmospheric oxygen, alone and in the presence of cysteine and ascorbic acid; pH 5.0 with acetate buffer. Molar ratio of cysteine to UCl_4 equals 3.5 : 1. Cysteine alone shows no inhibiting action on the oxidation at this ratio. △, UCl_4 alone; ○, UCl_4 and ascorbic acid; ▽, UCl_4, cysteine, and ascorbic acid.

In Fig. 1.27 the results of the experiment using cysteine are shown, and in Fig. 1.28 the results of the experiment with cysteine and ascorbic acid together are shown.

The effect of cysteine and glutathione appears to be due to the presence of SH groups in these substances, since other amino acids do not act in a similar manner. Cysteine is able to prevent the inhibition of enzyme activity by U_4. Apparently U_4 can complex with SH groups, although possibly not so strongly as does mercury, for example. However, it must again be emphasized that U_6 does not form complexes with SH groups. Since U_6 is the form of uranium in the body after the usual forms of exposure, a blocking of SH groups does not constitute a valid explanation for the toxicity of uranium. It is only after direct injection of U_4 into an animal that SH blocking might occur; but in this connection it is interesting that the toxicity of injected U_4 appears to be less than that of injected U_6.

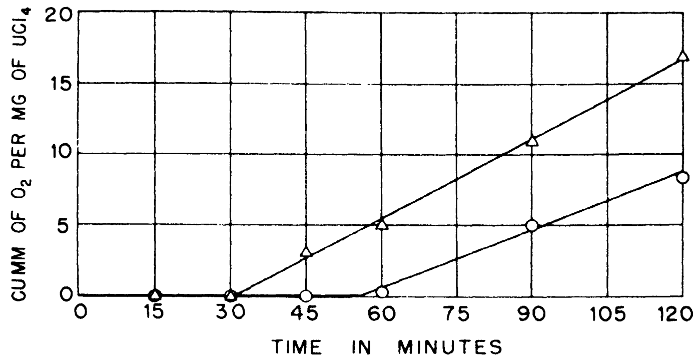

Fig. 1.29—Oxidation of UCl_4 by atmospheric oxygen, alone and in the presence of serum albumin; pH 5.0 with 0.1M acetate buffer. △, 9.9 mg of UCl_4; ○, 7.0 mg of UCl_4 in 0.4 per cent crystallized horse-serum albumin.

Serum albumin also has an effect of retarding the oxidation of U_4 in the presence of acetate buffer as is shown in Fig. 1.29. It is probable that this effect is at least partly explained by the insolubility of the U_4-albumin complex, since a precipitate was observed when the albumin was added to the U_4-buffer solution.

(d) Indicator-dye Studies of the Oxidation-Reduction Potential of the U_6,U_4 System. In this work methylene blue has been used, because of its convenience and because its oxidation-reduction potential lies reasonably close to that of the U_6,U_4 system. It is the potential of the latter system that is of importance in biochemical studies, since, as has been explained, other valence states of uranium cannot be present in the body in more than biochemically unimportant trace amounts.

It has been possible to cause oxidation of U_4 by methylene blue and reduction of U_6 by leucomethylene blue at pH values from 4 to 5 in the presence of acetate buffer, when oxygen was excluded from the system. The leucomethylene blue was prepared by just bleaching methylene blue with hydrosulfite, and then adding enough methylene blue to produce a very faint blue color. By roughly estimating the amount of methylene blue and leucomethylene blue present at equilibrium, it was possible to measure roughly the oxidation-reduction potential of the U_6,U_4 system at the given pH value. It was found by this method that, in acetate buffer, the potential E_0' for the U_6,U_4 system lies between 0.0 and −0.1 volt based on hydrogen at pH 5.5 to 6.0. This potential is somewhat lower than, but reasonably close to, the potential of methylene blue itself. Since methylene blue is reduced by cells (such as leucocytes) that it penetrates in the animal body, the possibility of U_6 reduction in the animal body must be considered. However, as will be discussed in Chap. 15, there is no good evidence that U_6 ever penetrates cells in the animal body in any appreciable amounts.

In conducting experiments with methylene blue it was found that the effect of the addition of citrate, which is a strong complexer for U_6 and U_4, was to slow the rate of the oxidation-reduction reactions very markedly. In addition to slowing the rate of the reactions, such a strong complexer could change the final equilibrium state very markedly if it complexed U_6 much more strongly than U_4 or vice versa. That there is a difference between the equilibrium potential of the U_6,U_4 system in citrate buffer and in acetate buffer will be seen subsequently.

It is probable that the effect of citrate in increasing the sluggishness of interaction between the U_6,U_4 system and the methylene blue-leucomethylene blue system is caused by a great retardation in the rate of the formation of U_5 by the reaction of U_4 with U_6. It is known definitely that in acetate buffer the rate of attainment of equilibrium potential at the platinum electrode is governed by the rate of attainment of equilibrium in the U_5 formation reaction.

2.4 Studies of the Oxidation-Reduction Potential of the U_6,U_4 System Using the Smooth Platinum Electrode as Indicator Electrode.*

(a) Potentials of the U_6,U_4 System in the Presence of Acetate Buffer. By using smooth platinum electrodes to study the oxidation-reduction properties of the U_6,U_4 system, results are obtained that are in essential agreement with those obtained from studies using methylene blue as an indicator. It has been possible to obtain constant potentials at the smooth platinum electrode using the U_6,U_4 system in acetate buffer, but considerable time is required for constant potentials to be attained. This point is illustrated in Fig. 1.30. It can be seen from this figure that the ratio of U_6 to U_4 also is of importance in determining the rate of attainment of fairly constant potentials. In general, a period of at least 45 min is required for the attainment of reasonably constant potentials after mixing the U_6 and the U_4 in acetate buffer.

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*For general information on the use of the smooth platinum electrode in measuring oxidation-reduction potentials, see bibliographic references 2, 4, and 5 at the end of this chapter. Specific references to investigators who have applied the platinum electrode to measurements of oxidation-reduction potentials of the U_6,U_4 system will be found in Appendix A to this chapter.

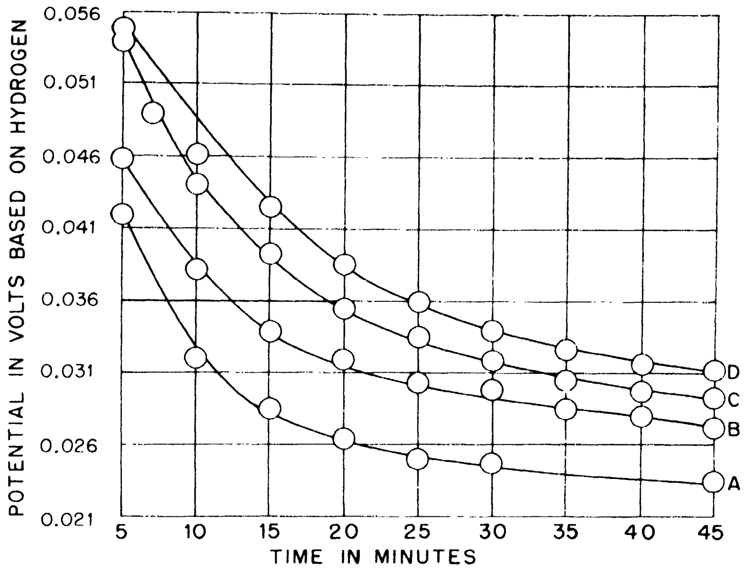

Fig. 1.30—Platinum-electrode potentials (converted to hydrogen base) of U_6,U_4 system under nitrogen plotted against time; pH 4.9 in acetate buffer. Ratios of U_6 to U_4: curve A, 0.87; curve B, 1.46; curve C, 1.75; curve D, 2.50. Total concentration of U_6 plus U_4 equals 0.002M.

Another factor that is of importance in determining the rate of attainment of constant potentials when the smooth platinum electrode is used to measure the redox potentials of U_6,U_4 systems is the total uranium concentration. An optimal concentration exists, with the ratio of U_6 to U_4 equal to 1, at which equilibrium is attained most rapidly. This is shown in Fig. 1.31, where the rate of change of potential is plotted against total uranium concentration. It is likely that different curves would be obtained if the ratio of U_6 to U_4 were varied. Since the rate of change of potential was measured as the potential attained during the first 5 min, the points plotted are considerably removed from the equilibration value.

It is not known why increasing the total uranium concentration above a certain optimal amount will cause retardation of the kinetics of oxidation-reduction reactions of the U_6,U_4 system at the smooth platinum electrode. It is possible, however, that polymerization of the U_4- and U_6-acetate complexes occurs, or that compound formation between U_6 and U_4 is the cause.

The cause for the slow rate of attainment of equilibrium potentials when the U_6,U_4 system is studied with the smooth platinum electrode appears to be slowness of establishment of the dismutation equilibrium involving U_5. Once this equilibrium has been established, in the absence of the electrode, it is possible to get instantaneous potentials that do not vary with time. The reaction poising the electrode appears to be the reaction involving equilibration between U_6 and U_5. This reaction, written for the free ions of uranium, is as follows:

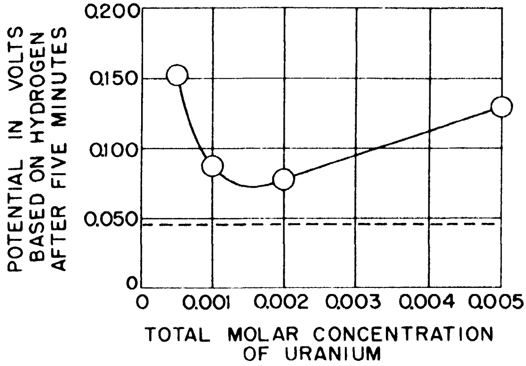

Fig. 1.31—Rate of attainment of equilibrium potential of the U_6,U_4 system in 0.05M acetate buffer, pH 5.0. Dashed line represents equilibrium potential.

$$UO_2^{++} + e \rightleftharpoons UO_2^{+}$$

Since the U_6,U_5 pair, the U_5,U_4 pair, and the U_6,U_4 pair are all in equilibrium when the final equilibrium of the U_6,U_4 system is established, the single poising reaction $UO_2^{++} + e \rightleftharpoons UO_2^{+}$, involving electron transfer between the system and the electrode, gives the U_6,U_4 potential. Proof of these statements is beyond the scope of this chapter.

Light affects the equilibrium of the U_6,U_4 system, but the effect is sufficiently small so that the results reported in this chapter, which were obtained in diffuse daylight, can also be regarded as essentially valid for darkness.

For a somewhat more detailed treatment of the kinetics of reactions establishing the potential at a smooth platinum electrode, as well as a treatment of the effect of light on the final potential (Becquerel effect), Appendix A to this chapter should be consulted.

In Fig. 1.32 are shown the results of measurements of the oxidation-reduction potentials (E_0' values) of the U_6,U_4 system in acetate buffer of pH 4.8 at various ratios of U_6 to U_4. The optimal concentration of total uranium was employed, and 40 min were allowed for the attainment of equilibrium after mixing the U_6 and U_4. This is a sufficiently long time for obtaining reasonably accurate results at the center portion of the curve, which is of the greatest importance, but it may not be long enough when the end portions of the curve are measured. The curve is obviously abnormal, in that the slope is too flat and the left end does not become negatively infinite. This abnormality may be partly explained, no doubt, by the sluggishness of the kinetics of the reaction that may have prevented attainment of equilibrium except in the center of the curve, and possibly also by the formation of aggregates of U_4 in the solution.

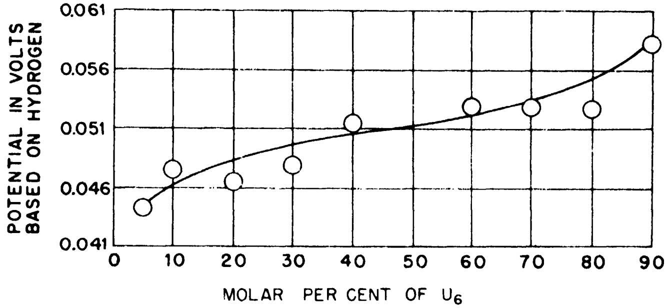

Fig. 1.32—Platinum-electrode potentials of the U_6,U_4 system at pH 4.8 in 0.1M acetate and 0.1M potassium chloride after 40 min. Total concentration of U_6 plus U_4 equals 0.004M.

The E_0' value read from the point on the curve where the concentrations of U_6 and U_4 are equal is 0.051 volt based on hydrogen, at pH 4.8 in acetate buffer. More will be said about the variation of E_0' with pH as determined by the smooth platinum electrode when the polarographic results are compared with results from the use of the smooth platinum electrode.

(b) Potentials of the U_6,U_4 System at the Smooth Platinum Electrode in the Presence of Bicarbonate. It was thought for some time that reproducible potentials could not be obtained from the U_6,U_4 system at the platinum electrode in the presence of bicarbonate. But recently it has been found that if pure CO_2 is passed into a solution of 0.5M bicarbonate to maintain a pH of about 7.3, the U_6,U_4 system will come to equilibrium at the platinum electrode fairly rapidly. If a pH of 8.5 is maintained by passing 5 per cent CO_2 in nitrogen into the solution, equilibrium is more slowly attained.

The result of this work is shown in Fig. 1.33. It was necessary to use 0.5M bicarbonate to keep the U_4 from precipitating.

It can be seen that the potential attained by the U_6,U_4 system in 0.5M bicarbonate at pH 7.3 is considerably higher than that attained in 0.05 to 0.10M acetate buffer. This is probably attributable to a greater complexing affinity of bicarbonate for U_6 than U_4. It can be shown that the equilibrium potential at equal concentrations of U_6 and U_4 depends upon the ratio of the dissociation constants of the U_6 and U_4 complexes that are present. This dependence is expressed quantitatively by the following equation:

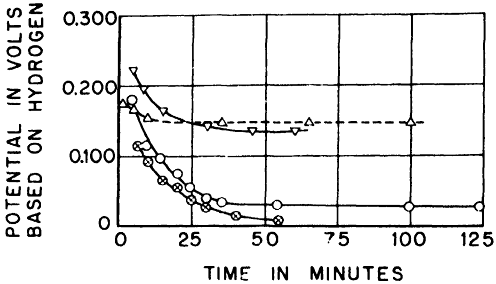

Fig. 1.33—Curves showing establishment of potential at platinum electrode of the U_4,U_6 system in bicarbonate-carbonic acid at different pH values. △ and ▽, 0.001M U_6, 0.001M U_4, 0.5M $NaHCO_3$, pH 7.3; ○ and ⊗, 0.001M U_6, 0.001M U_4, 0.5M $NaHCO_3$, pH 8.5.

$$\underset{6,4}{(E_0)_c} - \underset{6,4}{(E_0)_s} = 0.03 \log \frac{K_6}{K_4}$$

In this equation c refers to the complexed state, and s refers to the uncomplexed state of uranium. K_6 and K_4 are dissociation constants of the U_6 and U_4 complexes, respectively, to the free uranium ions.

It is not known whether the bicarbonate concentration affects the potential of the U_6,U_4 system at the platinum electrode or not, since it is impossible to maintain U_4 in a soluble state at the necessary concentration if the bicarbonate concentration is reduced much below 0.5M. Values of bicarbonate concentration above 0.5M cease to be interesting from a biochemical standpoint.

It can be seen from the data plotted in Fig. 1.33 that there is a change in equilibrium potential of about 0.1 volt per pH unit. In the absence of more accurate data and other information about the reaction, this slope cannot be interpreted on the basis of a simple reaction mechanism.

The potential of the U_6,U_4 system in bicarbonate indicates the possibility of reduction of the bicarbonate complex of U_6 in the body provided that this complex could enter cells. But as yet there is no evidence that the complex does enter cells, and the reduction of the complex in plasma would be very improbable.

(c) Potential of the U_6,U_4 System at the Smooth Platinum Electrode in Citrate Buffer. It has been found that the U_6,U_4 system is very sluggish in citrate buffer, but nevertheless reproducible potentials can be obtained using the platinum electrode, provided that sufficient time is allowed to elapse so that the equilibrium values may be obtained. There is very poor poising of the electrode, however, and considerable care must be exercised in taking the readings.

The results of the experiments with the U_6,U_4 system in citrate buffer are plotted in Fig. 1.34. It can be seen that there is no effect of citrate ion concentration of pH over the ranges investigated in the final potential, which is about −0.19 volt based on hydrogen in all cases. The final potential attained in all cases is so high that a reduction of U_6 citrate to U_4 citrate in cells would be predicted if it were not for the extreme sluggishness of the system. Again, however, there is no evidence as yet that the U_6-citrate complex would penetrate cells to any appreciable extent, and reduction of the U_6-citrate complex in plasma would be very unlikely. Moreover, the sluggishness of the system indicates that any possible reduction of the U_6-citrate complex in the body would be very slow in any case.

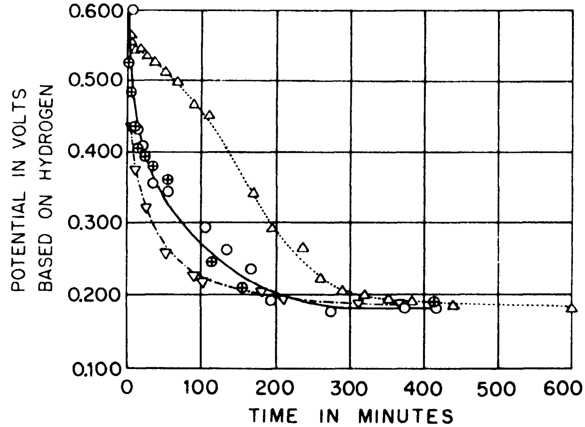

Fig. 1.34—Curves showing establishment of potential at platinum electrode of the U_6,U_4 system in citrate buffer. Essentially the same equilibrium potential is obtained in all cases. ○ and ⊗, 0.001M U_6, 0.001M U_4, 0.05M total citrate, pH 5.0 (poorly poised electrode); △, 0.001M U_6, 0.001M U_4, 0.1M total citrate, pH 5.0; ▽, 0.001M U_6, 0.001M U_4, 0.1M total citrate, pH 6.1.

The high equilibrium potential of the U_6,U_4 system in citrate indicates, as with bicarbonate, that citrate complexes U_6 more firmly than U_4.

2.5 Investigation of the Redox Potentials of the U_6,U_4 System under Various Conditions by Means of the Polarograph.* It is beyond the scope of this chapter to cover in detail all the work done at the University of Rochester on the polarography of uranium compounds. All that can be done is to outline briefly the work bearing on oxidation-reduction potentials.

Polarographic studies provide an independent means for investigating redox potentials of the U_6,U_4 system and yield results that confirm and greatly extend the information obtained by means of the platinum electrode. This polarographic work has been carried out for the most part by Tishkoff. The polarographic studies pertaining to oxidation-reduction studies are reported in some detail in an appendix to this chapter, which can be read with greatest profit only by those already well grounded in polarography.

The results stated in summary form are as follows: The polarographic spectrum of uranium at pH 3.6 in acetate buffer is illustrated in Fig. 1.35. Wave I is an anodic U_4,U_5 oxidation; wave II is a cathodic U_6,U_4 reduction; wave III presumably is a U_5,U_4 reduction wave; and wave IV is a U_4,U_3 reduction wave, partly combined with a U_5,U_4 reduction wave. At low pH (1 to 2) there are only two reduction waves, one for U_6,U_5, and one for a combined U_5,U_4 and U_4,U_3 reduction. This second wave therefore has twice the height of the first wave. This is illustrated in Fig. 1.36. Waves I, II, and IV appear to satisfy the various criteria of reversibility.

In the presence of bicarbonate, a strong complexer for U_6, the U_6,U_5 wave is moved to the right, indicating an increased difficulty in reduction. To understand this, it is assumed that, in obtaining the polarographic waves, sufficient potential is applied to the dropping-mercury electrode to cause reduction of the complexes of U_6 themselves, as well as reduction of the free ions. Since the free ions are much lower in concentration in the presence of strong complexers than the complexed uranium, the free ions contribute little if anything to the observed waves. It is therefore to be expected that different U_6 complexes will have somewhat different polarographic half-wave potentials.

In general, the polarographic half-wave potential for a reversible oxidation-reduction system is sufficiently close to the oxidation-reduction potential of the system so that the half-wave potential can be substituted for the oxidation-reduction potential without serious error. Of course, the system referred to must be the system actually

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*The reader unfamiliar with the general principles of polarography is referred to bibliographic references 1, 6, 7, and 8 at the end of this chapter. References to authors who have applied the polarographic method to a study of uranium compounds will be found in Appendix B to this chapter.

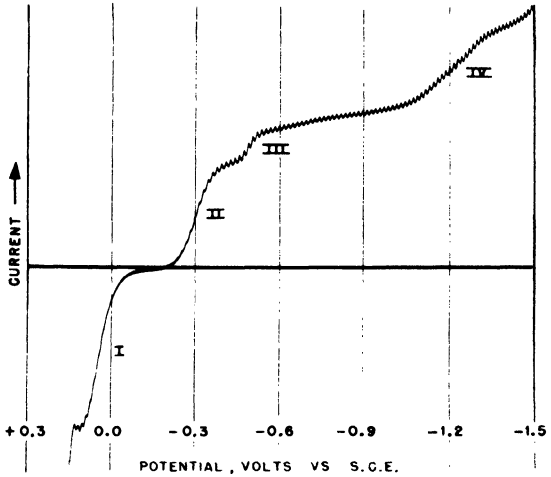


Fig. 1.35—Total polarographic spectrum of uranium in acetate medium at pH 3.6. U_6, 0.001M; U_4, 0.002M; sodium acetate, 0.054M; acetic acid, 0.3M; and KCl, 0.10M.

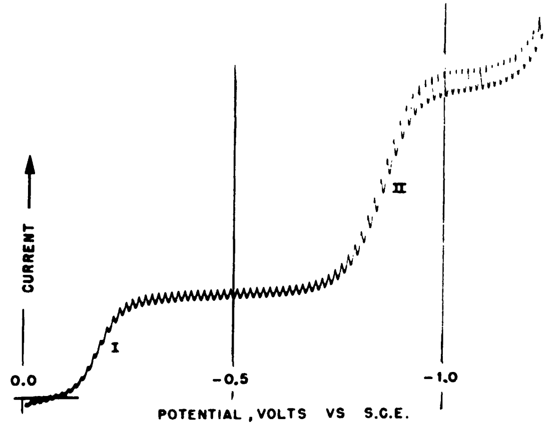


Fig. 1.36—Polarographic spectrum of uranium in 0.1N HCl solution, showing the U_6 cathodic wave I and the U_6,U_4 composite cathodic wave II.

taking part chemically in the reaction at the dropping-mercury electrode. It is sometimes possible to calculate a fictitious half-wave potential for an over-all process that actually goes on in distinct steps at the dropping-mercury electrode. Such a fictitious half-wave potential should also be close to the oxidation-reduction potential of the over-all process in question.

In Fig. 1.37, such a fictitious half-wave potential designated as $(E_{\frac{1}{2}})_{c3 \atop 6,4}$ for the U_6,U_4 system is plotted against pH in acetate buffer at a constant concentration of ionized acetate. The effect of change in ionized acetate concentration on the value of $(E_{\frac{1}{2}})_{c3 \atop 6,4}$ is thus kept constant, and the true effect of pH can be observed. $(E_{\frac{1}{2}})_{c3 \atop 6,4}$ is calculated from the observed half-wave potentials of the U_6,U_5 and U_5,U_4 systems by means of the equation:

$$(E_{\frac{1}{2}})_{c3 \atop 6,4} = \frac{(E_{\frac{1}{2}})_{c3 \atop 6,5} + (E_{\frac{1}{2}})_{c3 \atop 5,4}}{2}$$

where $(E_{\frac{1}{2}})_{c3 \atop 6,5}$ is the observed half-wave potential for the U_6,U_5 wave, and $(E_{\frac{1}{2}})_{c3 \atop 5,4}$ is the observed half-wave potential for the U_5,U_4 wave. This equation can be derived from elementary thermodynamical considerations involving the free-energy changes occurring in the processes under consideration. It is necessary to assume that the chemical form of U_5 is the same in the processes causing the U_6,U_5 and the U_5,U_4 waves, but this assumption is perfectly consistent with all the polarographic results bearing on the point.

In Fig. 1.37, the potential obtained by means of the smooth platinum electrode, using an optimal concentration of total uranium as already explained, also is plotted against pH. It can be seen that the polarographic potentials are in such close agreement with the platinum-electrode potentials that only one curve can be drawn for both sets of values. It is immaterial whether the platinum electrode is poised by the complexes or the free ions of the valence states of uranium in question, since the UO_2^{++} - UO^{++} system is in equilibrium with the system composed of U_6,U_4 complexes, so that the measured potential of both systems must be the same. The predominance of the complexes means that the concentrations of U_6 and U_4 complexes will be nearly the same when the concentration of total U_6 is made equal to the total concentration of U_4, and, in general, the concentrations of UO_2^{++} and UO^{++} will be different unless it happens that the degree of dissociation of the U_6 complex to free UO_2^{++} is exactly equal to the degree of dissociation of the U_4 complex to free UO^{++} ions. It follows that both the platinum electrode and the polarograph measure the potential of the system composed of complexes of U_6 and U_4, under the conditions prevailing in the experiments summarized in Fig. 1.37.

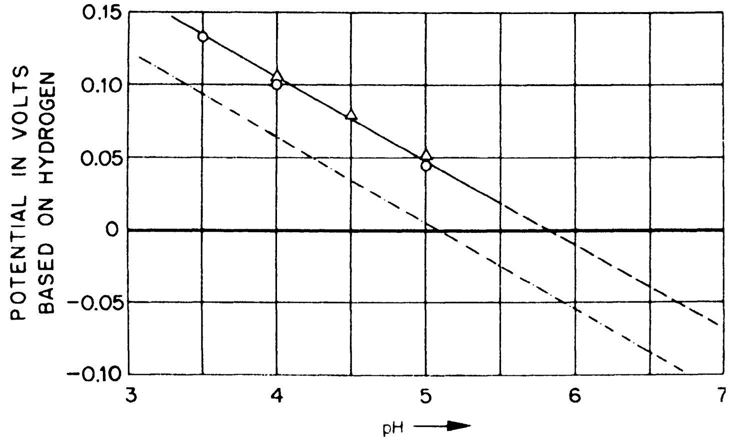


Fig. 1.37—Variation of potential of the U_6,U_4 system with pH at 0.05M concentration of ionized acetate as determined by the platinum electrode and the polarograph. ○, $E'_{0\ 6,4}$ from platinum-electrode data; total U, 0.004M; △, $E_{\frac{1}{2}\ 6,4}$, determined from the polarographic data by the formula $E_{\frac{1}{2}\ 6,4} = \dfrac{E_{\frac{1}{2}\ 6,5} + E_{\frac{1}{2}\ 4,5}}{2}$; total uranium concentration, 0.0001. Dot-and-dash line represents curve for $E'_{0\ 6,4}$ at 1M ionized acetate calculated from platinum-electrode data.

Under the conditions of the experiments illustrated in Fig. 1.37 by the polarographic data, the U_6 is complexed with three acetates, the U_4 with two acetates, and the U_5 with one acetate. The subscript c3 in the expression for the half-wave potentials refers to this state of conditions.

A polarographic study of the variation of $E_{\frac{1}{2}\ 6,5}$ and $E_{\frac{1}{2}\ 5,4}$ with pH has shown that the reduction of U_6 to U_5, whether as the free ion or as an acetate complex, is independent of pH, while the reduction of U_5 to U_4 whether free of ions or acetate complexes is dependent upon pH. Therefore the over-all reduction of U_6 to U_4 must depend upon pH, whether as free ions or as acetate complexes.

From examination of the upper curve in Fig. 1.37, it can be seen that an increase in pH makes reduction of U_6 to U_4 more difficult thermodynamically, and conversely makes oxidation of U_4 to U_6 easier.

Extrapolation of the upper curve to pH 7.0 yields an E_0' value of about −0.07 volt for the U_6,U_4 system. This value is such that, if U_6 were present in a living cell as $UO_2.3(O-\overset{\overset{O}{\|}}{C}-CH_3)$, at least partial reduction to U_4 would be likely, especially since it is known that methylene blue can be reduced by cells. But it must be stated once more that there is no convincing evidence that U_6 penetrates cells in the body in appreciable amounts, and that even if it could do so it would probably not be present as an acetate complex.

It has already been stated that at the particular concentration of acetate employed (0.05M), the dissociation of the acetate complexes of U_6 and U_4 were nearly equal, and hence the E_0' values for the UO_2^{++} - UO^{++} ion pair and for the acetate complexes of U_6 and U_4 would be nearly equal. But since U_6 is complexed with three acetates and U_4 with two acetates under the conditions of the polarographic experiments at an acetate concentration of about 0.05M or higher, it follows from elementary thermodynamical considerations that $\underset{6,4}{(E_{\frac{1}{2}})_{c3}}$ must vary with acetate concentration if the acetate concentration is gradually increased to values greater than 0.05M. The lower (broken) line in Fig. 1.37 shows the set of potentials to be expected theoretically from plotting variation of $\underset{6,4}{(E_{\frac{1}{2}})_{c3}}$ with pH at an ionized acetate concentration of 1M.

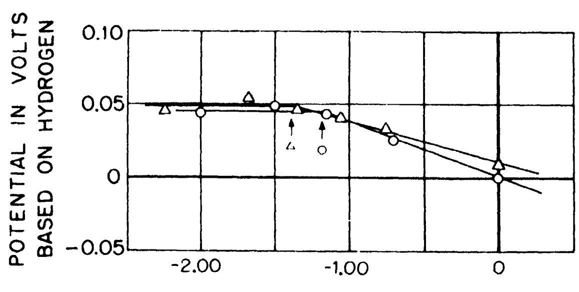
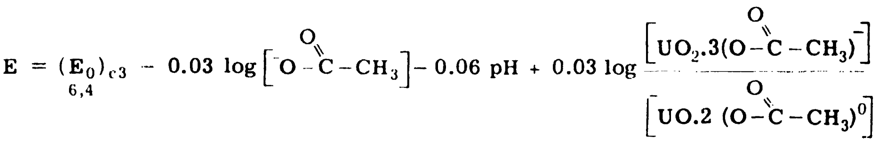


Fig. 1.38—Variation of potential of the U_6,U_4 system with ionized acetate concentration at pH 5.0 as determined by the platinum electrode and the polarograph. ○, $\underset{6,4}{E_0'}$, from platinum-electrode data; total uranium concentration, 0.004M; △, $\underset{6,4}{E_{\frac{1}{2}}}$, determined from polarographic data by the formula used previously; total uranium concentration, 0.0001M. Inflection points are as indicated.

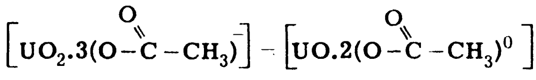
That such an acetate effect actually occurs is shown in Fig. 1.38, which was plotted from experimental data. Here potential is plotted against acetate concentration at constant pH. The results obtained from polarographic studies and platinum-electrode studies are in reasonably good agreement. Therefore it appears that the change effected from $UO_2.3(O-\overset{O}{\overset{\|}{C}}-CH_3)^-$ to $UO_2.2(O-\overset{O}{\overset{\|}{C}}-CH_3)^0$ occurs at a slightly higher acetate concentration in the case of the platinum electrode than in the case of the dropping-mercury electrode. This is because the uranium concentration is much higher in the case of the platinum electrode studies than in the case of the corresponding polarographic studies.

The complete equation showing variation of potential corresponding to the U_6,U_4 system with pH, acetate concentration, and ratio of U_6 to U_4 is as follows:



$$E = \underset{6,4}{(E_0)_{c3}} - 0.03\log\left[\overset{-}{O}-\overset{O}{\overset{\|}{C}}-CH_3\right] - 0.06\,pH + 0.03\log\frac{\left[UO_2.3(O-\overset{O}{\overset{\|}{C}}-CH_3)\right]^-}{\left[UO.2\,(O-\overset{O}{\overset{\|}{C}}-CH_3)^0\right]}$$

The subscript c3 refers to the fact that three acetates are complexed with U_6. When



$$\left[UO_2.3(O-\overset{O}{\overset{\|}{C}}-CH_3)\right]^- = \left[UO.2(O-\overset{O}{\overset{\|}{C}}-CH_3)^0\right]$$

the last term drops out, and E is measured by the calculated value of $\underset{6,4}{(E_{\frac{1}{2}})_{c3}}$ if the polarograph is employed, or by the observed value of the potential if the platinum electrode is used, at the specified pH and acetate concentration. The latter value will be designated as $\underset{6,4}{(E_0'')_{c3}}$.

$\underset{6,4}{(E_0)_{c3}}$ is the standard potential of the above system as ordinarily defined and can be calculated by making the appropriate substitutions into the above equation.

It has been found from polarographic studies that, if the concentration of acetate is less than 0.05M, at total uranium concentrations of 0.001M or less, the U_6 is complexed with two acetates instead of three, and U_5 is complexed with zero acetates, but the U_4 is still complexed with two acetates as at higher acetate concentrations. Under such conditions the curve showing the variation of $\underset{6,4}{(E_{\frac{1}{2}})_{c2}}$ with pH is independent of acetate concentration until so little acetate is present that the U_6 and U_4 cannot be complexed entirely as biacetates. The subscript c2 refers to the number of acetates complexed, i.e., 2 with U_6, 0 with U_5, and 2 with U_4.

Figure 1.39 shows the results of polarographic and smooth-platinum-electrode studies of the variation of the measured potential at

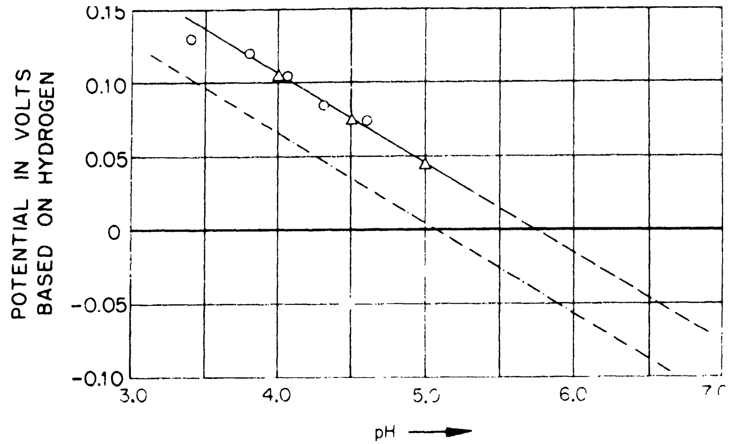


Fig. 1.39—Variation of potential of the U_6,U_4 system with pH at 0.01M ionized acetate concentration as determined by the platinum electrode and the polarograph. ○, $E'_{0_{6,4}}$ from platinum-electrode data; total uranium concentration, 0.004M; △, $E_{\frac{1}{2}_{6,4}}$ determined from polarographic data by the formula used previously; – – – –, curve calculated for $E_{\frac{1}{2}_{6,4}}$ with any complex. Note: In practice, points at values above pH 4.0 (approximately) would be impossible to determine because of insolubility of U_4; hence the value at pH 7.0 is theoretical.

equal U_6 and U_4 concentrations of the U_6,U_4 system in 0.01M ionized acetate at pH 5.0. The results obtained from the platinum-electrode studies are indistinguishable from results obtained from the polarographic studies. Extrapolation of the curve to pH 7.0 gives a potential $(E'_{0_{6,4}})_{c2}$ or $(E_{\frac{1}{2}_{6,4}})_{c2}$ of −0.07 volt, the same value obtained under the conditions of the experiments summarized in Fig. 1.37.

It may be asked why the upper curves in Figs. 1.37 and 1.39 are practically identical. To understand this, the platinum-electrode and polarographic data must be considered separately. In the case of the polarographic data, the upper curve in Fig. 1.39 illustrates the case when two acetates are complexed with U_6 and two with U_4, and in Fig. 1.37 the upper curve illustrates the case where three acetates are complexed with U_6 and two with U_4. At the acetate concentration corresponding to the upper curve in Fig. 1.37 (0.05M ionized acetate) the transition from $UO_2.2(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^0$ to $UO_2.3(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^-$ is just occurring; i.e., the point where $UO_2.2(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^0$ and $UO_2.3(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^-$ are in equilibrium is represented by 0.05M acetate, so that the same curve will be obtained for both situations.

In the case of the platinum-electrode data, the upper curve in Fig. 1.37 and the upper curve in Fig. 1.39 probably both correspond to the case where two acetates are complexed with U_6 and two with U_4. This is because a much higher concentration of total uranium was employed in the platinum-electrode studies than in the polarographic studies. The concentration of acetate at which the change from $UO_2.2(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^0$ to $UO_2.3(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^-$ occurs depends upon the ratio of acetate concentration to uranium concentration. In the case of the platinum-electrode studies, this ratio is probably not quite high enough to cause a transition from $UO_2.2(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^0$ to $UO_2.3(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^-$ at an ionized acetate concentration of 0.05M.

The general equation describing the behavior of the U_6,U_4 system in acetate buffer, under the conditions of the experiments illustrated in Fig. 1.39 is as follows:

$$E = (E_0)_{\substack{c2\\6,4}} - 0.06\ pH + 0.03 \log \frac{\left[UO_2.2(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^0\right]}{\left[UO.2(O-\overset{\overset{O}{\backslash\backslash}}{C}-CH_3)^0\right]}$$

The subscript 2 refers to the fact that two acetates are complexed with U_6. $(E_0)_{\substack{c2\\6,4}}$ is the standard potential of the system under the conditions prevailing in the experiments summarized in the top curve of Fig. 1.39. E can be measured by the calculated $(E_{\frac{1}{2}})_{\substack{c2\\6,4}}$ value if the polarographic method is used, or from the observed $(E_0')_{\substack{c2\\6,4}}$ if the platinum electrode is used. The standard potential $(E_0)_{\substack{c2\\6,4}}$ can be obtained by making the appropriate substitutions into the above equation.

In Fig. 1.39 the lower (broken) line shows the calculated variation in potential against pH $\underset{6,4}{(E_{\frac{1}{2}})_s}$ for the free ions in the absence of any complexer. The subscript s refers to the fact that the free ions of U_6 and U_4 are being considered. This curve was verified from polarographic studies from pH 2 to pH 4.5 but not at higher pH values owing to the great insolubility of uncomplexed U_4 at pH values higher than 4.5. The extrapolated value for $\underset{6,4}{(E_{\frac{1}{2}})_s}$ at pH 7.0 is about −0.12 volt based on hydrogen. The equation describing the behavior of the free ion system is as follows:

$$E = \underset{6,4}{(E_0)_s} - 0.06\ \text{pH} + 0.03 \log \frac{[UO_2^{++}]}{[UO^{++}]}$$

E can be measured as $\underset{6,4}{(E_{\frac{1}{2}})_s}$ calculated from polarographic data, or as $\underset{6,4}{(E_0')_s}$ determined by means of the platinum electrode. The standard potential $\underset{6,4}{(E_0)_s}$ can be calculated by making the appropriate substitutions in the above equation.

Although monoacetates of U_6 and U_4 are postulated, these compounds would become important only in systems containing low ratios of acetate to uranium, and no experimental work has been done to try to investigate the potentials of such systems. Something might be done, however, by calculation, using constants and data already available.

If the standard potential $\underset{6,4}{(E_0)_s}$ for the uncomplexed UO_2^{++} - UO^{++} system is calculated from the equation above, using polarographic data, with acid solutions of UO_2^{++} and UO^{++} in the neighborhood of pH 1.0, a value of 0.324 volt is obtained, which is in good agreement with the value of +0.328 volt reported from the National Bureau of Standards[3] in spite of the fact that the data in the two cases were obtained by different experimental methods.

$\underset{6,4}{(E_0)_{c3}}$ and $\underset{6,4}{(E_0)_{c2}}$ were calculated from the curves in Figs. 1.37 and 1.39 by choosing a potential and its corresponding pH and substituting into the appropriate equations (already given). As already stated, $\underset{6,4}{(E_0)_s}$ was calculated from polarographic data that are not included in this chapter.

In brief summary, polarographic studies show that either two or three acetate groups can complex with U_6, depending upon the acetate concentration, and that two acetate groups generally complex with U_4.

Table 1.4—Standard Potentials for the Various U_6,U_4 Systems

No. of acetates complexed with U_6	No. of acetates complexed with U_4	Standard potential, volts
3	2	$\underset{6,4}{(E_0)}_{c3} = +0.302$ Polarographic data
2	2	$\underset{6,4}{(E_0)} = +0.346$ Polarographic and platinum-electrode data
0	0	$\underset{6,4}{(E_0)}_{s} = 0.324$ Polarographic data

U_5 generally carries one acetate. At low ratios of acetate to uranium it is postulated that monoacetates of U_6 and U_4 occur. The general procedure for making such investigations is outlined by Kolthoff and Lingane.[1] A more detailed treatment will be found in Appendix B of this chapter. Table 1.5 summarizes these results.

Table 1.5

Acetate concentration	No. of acetates complexed with U_6	No. of acetates complexed with U_4	No. of acetates complexed with U_5
0.05M or higher	3	2	1
0.01–0.04M	2	2	1

This table applies when the total uranium concentration is 0.0001 to 0.004M. Concentrations outside this range have not been investigated.

The dissociation constants of the most important acetate complexes of uranium also have been determined through polarographic studies (see Appendix B to this chapter). These constants are summarized in Table 1.6. They do not have any immediate biological application that is apparent.

Since some of these complexes might have a tendency to become hydrated and then ionize in an acidic manner, the ionic charges assigned to the complexes are not necessarily correct in all cases.

In this chapter a considerable amount of information has been presented about U_6 and U_4 in acetate solutions. Much of this material is not directly applicable to studies of uranium in the body, but it serves as a model study of the behavior of uranium with one class of complexer and gives a general idea of what oxidation-reduction properties are to be expected from the U_6,U_4 system. Further applications of the results of studies of the oxidation-reduction properties of the U_6,U_4 system in an investigation of the oxidation level of uranium in the body will be found in Chap. 13.

Table 1.6—Dissociation Constants of Uranium Acetate Complexes*

Equilibrium	Constant	Valence of uranium
$UO_2(Ac)_3^- \rightleftharpoons UO_2^{++} + 3Ac^-$	$K_{3-0} = 5.5 \times 10^{-7}$	+6
$UO_2(Ac)_2^0 \rightleftharpoons UO_2^{++} + 2Ac^-$	$K_{2-0} = 2.2 \times 10^{-5}$	+6
$UO_2(Ac)_3^- \rightleftharpoons UO_2(Ac)_2^0 + Ac^-$	$K_{3-2} = 2.6 \times 10^{-2}$	+6
$UO(Ac)_2^0 \rightleftharpoons UO^{++} + 2Ac^-$	$K_{2-0} = 2.4 \times 10^{-6}$	+4
$UO_2(Ac)^0 \rightleftharpoons UO_2^+ + Ac^-$	$K_{1-0} = 2.6 \times 10^{-2}$	+5

*Throughout this book the symbol "Ac" represents the acetate group and not the element actinium.

2.6 Additional Physical Chemistry of Uranium Compounds in Solution. A subject of interest is the possibility of polymerization of uranium compounds in solution. Evidence submitted by Sunier using the McBain sintered-glass diffusion cell indicates that in bicarbonate solution U_6 either is not polymerized at all or, if polymerized, is not larger on the average than a dimer.

3. SUMMARY

1. The preparation and properties of some uranium compounds important in toxicological work have been given.

2. An outline of the aspects of the chemistry of uranium compounds that apply to biochemical and toxicological studies has been given. This outline includes the following topics:

(a) The chemistry of hexavalent uranium with particular reference to the formation of complexes with various materials in solution.

(b) A brief description of the chemistry of tetravalent uranium with particular regard to a comparison of its chemistry with the chemistry of U_6.

(c) A study of the oxidation-reduction properties of the hexavalent-tetravalent uranium system, with particular regard to the effect of complexers, pH, and the role of pentavalent uranium.

(d) Dissociation constants of various acetate complexes of U_6 and U_4 have been presented.

(e) The problem of polymerization of uranium compounds in solution has been touched upon.

BIBLIOGRAPHY

1. I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, 1941.
2. L. Michaelis, Cold Spring Harbor Symposia Quant. Biol., 7: 33 (1939).
3. J. K. Taylor and E. R. Smith, Nat. Bur. of Standards (U. S.), Rept. A1972, Aug. 29, 1944.
4. L. Michaelis, "Oxidation-Reduction Potentials," translated by L. B. Flexner, J. B. Lippincott Company, Philadelphia, 1930.
5. L. Michaelis and M. P. Schubert, Chem. Revs., 22: 437 (1938).
6. O. H. Muller, "The Polarographic Method of Analysis," published by the Journal of Chemical Education, Easton, Pa., 1941.
7. O. H. Muller, Cold Spring Harbor Symposia Quant. Biol., 7: 59 (1939).
8. O. H. Muller, "Physical Methods of Organic Chemistry," edited by A. Weissberger, Vol. II, Chap. XXIII, Interscience Publishers, Inc., New York, 1946.

APPENDIX A

By G. H. Tishkoff

H. G. Heal[1] has presented a theory to explain the kinetics of the reactions occurring at the platinum electrode in the case of the U_6,U_4 system and also to explain the Becquerel effect.* His theory, which agrees quite well with the experimental data, is based upon the existence of U_5, which is formed by reaction of U_6 with U_4. He states in effect that the electrode-poising valence pair is the U_6,U_5 pair, not the U_5,U_4 pair, or the U_6,U_4 pair. It is very easy to see that the potential given by the U_6,U_5 pair in a solution containing U_6,U_5, and U_4 must be the same as the potential theoretically attributable to the U_6,U_4 pair, since equilibrium between these two pairs is established by the dismutation reaction. The latter reaction can be written in a reverse manner as a reaction of formation of U_5 as follows:

$$H_2O + UO_2^{++} + UO^{++} = 2UO_2^+ + 2H^+$$

This particular equation applied to noncomplexing media (for instance, 0.05N HCl).

The equilibrium constant for the reaction, K_H†, is determined by the following equation:

$$K_H = \frac{[UO_2^+]^2\,[H^+]^2}{[UO_2^{++}]\,[UO^{++}]}$$

The concentration of UO_2^+ can be expressed as follows:

$$[UO_2^+] = \frac{\sqrt{K_H\,[UO_2^{++}]\,[UO^{++}]}}{[H^+]}$$

---

*The Becquerel effect is the name given to the phenomenon whereby the equilibrium potential of the U_6,U_4 system at a platinum electrode is shifted under the influence of light. It was apparently first observed by Baur,[2] using uranyl nitrate solutions.

†The subscript H in the term K_H refers to the fact that the reaction for UO_2^+ formation is written so as to involve hydrogen ions.

The equation for the potential set up by the U_6,U_5 pair at the platinum electrode is as follows:

$$E = (E_0)_{\underset{6,5}{s}} + \frac{RT}{F} \ln \frac{[UO_2^{++}]}{[UO_2^+]}$$

where all potentials are based upon the standard hydrogen electrode, taking the potential of the latter as equal to zero. Substituting the expression given above for $[UO_2^+]$, the following equation is obtained:

$$E = (E_0)_{\underset{6,5}{s}} + \frac{RT}{F} \ln \frac{[UO_2^{++}][H^+]}{\sqrt{K_H[UO_2^{++}][UO^{++}]}}$$

Simplifying,

$$E = (E_0)_{\underset{6,5}{s}} + \frac{RT}{2F} \ln \frac{[UO_2^{++}][H^+]^2}{K_H[UO^{++}]}$$

The term ln $(1/K_H)$ can be combined with the constant term $E_{\underset{6,5}{0}}$ as follows:

$$E = (E_0)_{\underset{6,5}{s}} - \frac{RT}{2F} \ln K_H + \frac{RT}{2F} \ln \frac{[UO_2^{++}][H^+]^2}{[UO^{++}]}$$

This equation has the same form as the equation

$$E = (E_0)_{\underset{6,4}{s}} + \frac{RT}{2F} \ln \frac{[UO_2^{++}][H^+]^2}{[UO^{++}]}$$

The E values are identical at equilibrium conditions as has been explained, and the last terms are also identical. Therefore

$$(E_0)_{\underset{6,4}{s}} = (E_0)_{\underset{6,5}{s}} - \frac{RT}{2F} \ln K_H$$

The question arises as to why the U_6,U_5 system alone should establish the potential, without the U_5,U_4 system contributing anything. Heal states that the oxidation of U_4 involves hydroxyl ions. In acid medium the concentration of OH^- would be sufficiently small so that this reaction would occur very slowly, and for this reason the U_6,U_4 reaction would not be rate-determining. Heal concludes the U_6,U_5 reaction may be expected to occur much more rapidly than the U_6,U_4 reaction, and will consequently determine the electrode potential. This statement would imply that the U_6,U_4 reaction occurs by a direct 2-electron change. An explanation that would be more in accord with Michaelis's theory of 1-electron step reactions is as follows:

The reduction of U_6 to U_4 or the reverse process can be written as two individual 1-electron step equilibriums

$$UO_2^{++} + e \rightleftharpoons UO_2^{+} \tag{1}$$

$$H_2O + UO_2^{+} + e \rightleftharpoons UO^{++} + 2OH^{-} \tag{2}$$

Equations 1 and 2 are known to be reversible and well-established reactions from polarographic investigations. Equation 2 shows that the oxidation of UO^{++} would be slow at low pH values, since the concentration of OH^- is very small. However, Eq. 2 could be replaced by

$$2H^{+} + UO_2^{+} + e \rightleftharpoons UO^{++} + H_2O \tag{2a}$$

According to Eq. 2a the reduction of UO_2^+ would be favored at low pH values, and equilibrium should be readily established. This is contrary to the argument proposed by Heal.

It can be seen that both Eqs. 2 and 2a involve trimolecular collisions on the platinum surface. The rate of attainment of equilibrium of such reactions would presumably be very small compared to the U_6,U_5 equilibrium, which involves collision of only 1 molecule with the platinum surface, and hence the latter reaction can be expected to predominate.

Briefly summing up, it has been proposed that the equilibrium potential of the U_6,U_4 system at an inert electrode is attained through the U_6,U_5 potential and is equal to the summation of this potential with a function of the formation constant. It has also been proposed (from a theoretical and experimental basis) that the reaction kinetics must involve the intermediate U_5.

Little has been said in regard to the mechanism of the dismutation reaction. It is possible to write three reactions, any of which would express the over-all results as follows:

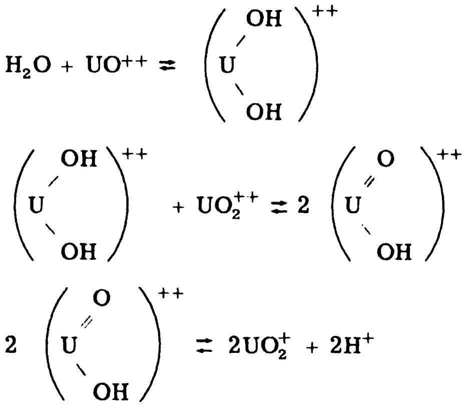
(i) $H_2O + UO_2^{++} + UO^{++} \rightleftharpoons 2UO_2^{+} + 2H^{+}$

(ii) $2OH^{-} + UO_2^{++} + UO^{++} \rightleftharpoons 2UO_2^{+} + H_2O$

(iii) $OH^{-} + UO_2^{++} + UO^{++} \rightleftharpoons 2UO_2^{+} + H^{+}$

As an over-all reaction, Eq. (i) would correspond to conditions where the platinum-electrode potential is found to depend upon the hydrogen-ion concentration taken to the second power, provided that complexing ions are absent. This can be readily seen if it is recalled that the reaction $UO_2^{++} + e \rightleftharpoons UO_2^+$ is independent of hydrogen-ion concentration, and that the over-all reaction at the platinum electrode is made up of the latter reaction and the dismutation reaction added together. Therefore if the over-all reaction depends upon $[H^+]$ taken to the second power, the dismutation reaction must depend upon hydrogen-ion concentration taken to the second power.

As Heal points out, the process of dismutation depends no doubt upon consecutive reactions, since the three over-all reactions given above are too complicated to be probable from a kinetic standpoint. A set of reactions that might explain the formation-dismutation reaction in regions where the formation-dismutation equilibrium depends upon the square of the hydrogen-ion concentration is as follows:



$$H_2O + UO^{++} \rightleftharpoons \left(\mathrm{U}\begin{array}{l}\diagup \mathrm{OH}\\ \diagdown \mathrm{OH}\end{array}\right)^{++}$$

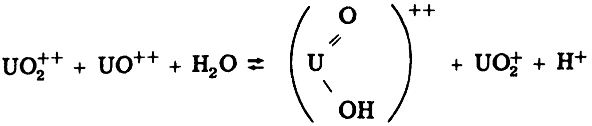
$$\left(\mathrm{U}\begin{array}{l}\diagup \mathrm{OH}\\ \diagdown \mathrm{OH}\end{array}\right)^{++} + UO_2^{++} \rightleftharpoons 2\left(\mathrm{U}\begin{array}{l}/\!/ \mathrm{O}\\ \diagdown \mathrm{OH}\end{array}\right)^{++}$$

$$2\left(\mathrm{U}\begin{array}{l}/\!/ \mathrm{O}\\ \diagdown \mathrm{OH}\end{array}\right)^{++} \rightleftharpoons 2UO_2^+ + 2H^+$$

Summing these reactions, Eq. (i) is obtained:

$$H_2O + UO_2^{++} + UO^{++} \rightleftharpoons 2UO_2^+ + 2H^+$$

Actually it has been found in this laboratory that the potential at the platinum electrode, and hence the formation-dismutation reaction, depends upon the hydrogen-ion concentration squared under conditions where U_6 and U_4 are complexed with two acetates each, and also when U_6 is complexed with three acetates and U_4 with two acetates. In acid solutions from pH 2.5 to 3.5 where the uranium is present largely in the complexed condition, the reaction appears to depend upon the first power of the hydrogen-ion concentration. It is possible to write a dismutation mechanism for these conditions as follows:



$$UO_2^{++} + UO^{++} + H_2O \rightleftharpoons \left(U \begin{array}{l} {/\!/}\,O \\ \backslash\, OH \end{array} \right)^{++} + UO_2^{+} + H^{+}$$

where the dismutation constant depends upon the first power of the hydrogen-ion concentration.

It should be pointed out that Heal states that the formation-dismutation reaction in very strongly acid solution (about 1M) depends upon the first power of the hydrogen-ion concentration, while the over-all electrode reaction depends upon the fourth power of the hydrogen-ion concentration. If this is true, it follows that the U_6,U_5 reaction when doubled must depend upon the cube of the hydrogen-ion concentration, in order that the over-all reaction can be written as the sum of the U_6,U_5 electrode reaction plus the formation-dismutation reaction. But it is not very plausible that the U_6,U_5 reaction when doubled should depend upon the cube of the hydrogen-ion concentration; furthermore, Heal states that the U_6,U_5 electrode reaction is independent of pH even in strongly acid solution. Thus there is an inconsistency in Heal's views.

In the case that two acetates are complexed with U_6 and two with U_4, it is found that no acetates are complexed with U_5. Since two hydrogen atoms are involved in the dismutation reaction in this case, the over-all reaction must be written as follows:

$$H_2O + UO_2(Ac)_2 + UO(Ac)_2 \rightleftharpoons 2UO_2^{+} + 4Ac^{-} + 2H^{+} \qquad (3)$$

This shows that the dismutation equilibrium depends upon the acetate concentration taken to the fourth power. Since the over-all reaction at either the platinum or mercury electrode does not depend upon the acetate concentration at all under these conditions, it follows that the U_6,U_5 reaction at the electrode when doubled must depend inversely upon the fourth power of the acetate-ion concentration, so that the acetate effect cancels out. That this is so can be seen from the following reaction:

$$2UO_2^{+} + 4Ac^{-} \rightleftharpoons 2UO_2(Ac)_2 + 2e \qquad (4)$$

Adding Eqs. 3 and 4, the over-all reaction is obtained. This is the reaction found experimentally to correspond to the over-all reaction.

$$UO_2(Ac)_2 + 2H^{+} + 2e \rightleftharpoons UO(Ac)_2 + H_2O$$

Under conditions where three acetates are complexed with U_6 and two with U_4, it is found that one acetate is complexed with U_5, and the over-all reaction depends upon the square of the hydrogen-ion concentration and the acetate concentration taken to the first power. Under these conditions the U_6,U_5 formation-dismutation reaction and the U_6,U_5 electrode reactions are written as follows:

$$UO_2(Ac)_3^- + UO(Ac)_2 + H_2O \rightleftharpoons 2UO_2(Ac) + 2H^+ + 3Ac^-$$

and

$$4Ac^- + 2UO_2(Ac) \rightleftharpoons 2UO_2(Ac)_3^- + 2e$$

Adding, the following over-all reaction is obtained, which corresponds to the U_6,U_4 potential:

$$UO_2(Ac)_3^- + 2H^+ + 2e \rightleftharpoons UO(Ac)_2 + Ac^- + H_2O$$

This reaction corresponds to the experimental facts, as has already been pointed out.

From the thermodynamic viewpoint, Eqs. (i) and (ii) are applicable to describe the over-all formation-dismutation reactions and will be employed henceforth.

Kinetics of the Formation-Disproportionation Reaction and the Becquerel Effect. It has been stated or clearly implied that the factor determining the rate of establishing the final equilibrium potential of the U_6,U_4 system at the smooth platinum electrode is the rate of the formation-disproportionation reaction involving U_5, whether complexing or noncomplexing media are considered. The initial reaction $U_6 + e = U_5$, which poises the electrode, is very rapid since it involves collision of only a single molecular species with the platinum surface and thus is not rate determining. The hypothetical reaction $U_5 + e = U_4$ evidently does not occur at a measurable rate at the platinum surface. It will be shown also that the Becquerel effect depends upon the action of light in shifting the equilibrium of the formation-dismutation reaction. As will be seen, the general equation describing the change of the U_6,U_4 potential at the platinum electrode with time until final equilibrium is established is the same whether the solutions are in darkness or in bright light. Furthermore, the same general type of equation applies for the potential change that occurs upon suddenly illuminating a solution that has been in the dark.

The above-mentioned time-potential curves have been studied in some detail by Titlestad[3] under various concentrations of uranium and acidity and under different light intensities. Schiller,[4] Truempler,[5] Swensson,[6] and Ghosh[7] likewise have contributed extensively to the existing knowledge of the mechanism of the U_6,U_4 potential at the platinum electrode. The mechanisms that were proposed by these investigators involved the postulation of the intermediates U_3 or U_5. A quantitative explanation of the time-potential curves will now be formulated which agrees with all aspects of the experimental evidence and is founded upon the formation-dismutation reaction

$$U_6 + U_4 \rightleftarrows 2U_5$$

The hypothesis is essentially a corollary of the hypothesis presented by Heal to explain the Becquerel effect.

A suitable equation based on this hypothesis can be derived to describe the experimentally determined time-potential curves, some of which already have been presented in the main part of the chapter. Let the potential readings be recorded from the instant U_6 and U_4 are introduced into the cell (i.e., sufficient time has not elapsed for an equilibrium concentration of U_5 to be established). Then when $t = 0$, $[U_5] = 0$.

Let C_{max} = concentration of UO_2^+ (U_5) when the potential has become steady.
C = instantaneous concentration of UO_2^+ at any point on curve.
t = time from start of measurements.

It is assumed in the following derivation that the concentrations of U_6 and U_4 are not appreciably decreased as a result of the formation reaction of UO_2^+. Another assumption is that the reaction for the dismutation of U_5 is of second order, as was previously pointed out.

At equilibrium the rate of disproportionation of U_5 is given by kC_{max}^2, where k is the velocity constant. Since the system is at equilibrium the expression is also equal to the rate of formation of U_5. The instantaneous rate of disproportionation at any point on the curve is kC^2. The net rate of formation is given as the difference of the above two expressions, or

$$\frac{dC}{dt} = k(C_{max}^2 - C^2) \tag{5}$$

On integration of this relation, the following expression is derived:

$$t + \text{const.} = \frac{1}{2C_{max}\,k} \ln \left(\frac{C_{max} + C}{C_{max} - C}\right)$$

when $t = 0$, $C = 0$; therefore, const. = 0; or

$$t = \frac{1}{2C_{max}\,k} \ln\left(\frac{C_{max} + C}{C_{max} - C}\right) \tag{6}$$

If our conclusions concerning the U_6,U_5 potential are valid, then the potential behavior at any point is given by the Nernst equation, where E_i refers to the instantaneous potential (based on hydrogen):

$$E_i - \underset{6,5}{(E_0)_s} = \frac{RT}{F} \ln \left\{\frac{[UO_2^{++}]}{C}\right\}$$

and

$$Ce^{F[E_i - \underset{6,5}{(E_0)_s}]/RT} = [UO_2^{++}] \tag{7}$$

At the steady state,

$$E - \underset{6,5}{(E_0)_s} = \frac{RT}{F} \ln \left\{\frac{[UO_2^{++}]}{C_{max}}\right\}$$

and

$$C_{max}\, e^{F[E - \underset{6,5}{(E_0)_s}]/RT} = [UO_2^{++}] \tag{8}$$

Since it is assumed that the concentrations of UO_2^{++} and UO^{++} are constant, Eqs. 7 and 8 may be equated and

$$Ce^{F[E_i - \underset{6,5}{(E_0)_s}]/RT} = C_{max}\, e^{F[E - \underset{6,5}{(E_0)_s}]/RT}$$

It follows that

$$C = C_{max}\, e^{F(E_i - E)/RT}$$

On substitution into Eq. 6,

$$t = \frac{1}{2C_{max}\,k} \ln\left[\frac{1 + e^{F(E - E_i)/RT}}{1 - e^{F(E - E_i)/RT}}\right] \tag{9}$$

Equation 9 describes the time-potential curves for a system not at equilibrium at $t = 0$. The relation finally derived can be shown in the following manner to be of the type $y = k - Ae^{-ct}$, which was employed to describe empirically the observed time-potential curves. From Eq. 9 it is apparent that

$$e^{2C_{\max}kt} = \frac{1 + e^{F(E - E_i)/RT}}{1 - e^{F(E - E_i)/RT}}$$

It can be shown that

$$\frac{e^{2C_{\max}kt} - 1}{e^{2C_{\max}kt} + 1} = e^{F(E - E_i)/RT}$$

Let $2C_{\max}\,k = M$; then

$$\frac{e^{Mt} - 1}{e^{Mt} + 1} = e^{F(E - E_i)/RT} = \frac{1 - e^{-Mt}}{1 + e^{-Mt}} \qquad (10)$$

Taking the logarithm of Eq. 10 and rearranging, it is found that

$$\begin{aligned} E_i &= E - \frac{RT}{F} \ln \left[\frac{1 - e^{-Mt}}{1 + e^{-Mt}}\right] \\ E_i &= E - \frac{RT}{F} \left[\ln (1 - e^{-Mt}) - \ln (1 + e^{-Mt})\right] \end{aligned} \qquad (11)$$

By expanding the logarithmic terms into an exponential series, it follows that

$$E_i = E - \frac{RT}{F} \left[-2e^{-Mt} - \frac{2e^{-3Mt}}{3} - \frac{2e^{-5Mt}}{5} \dots\right]$$

$$E_i = E + \frac{2RT}{F} \left[e^{-Mt} + \frac{e^{-3Mt}}{3} + \frac{e^{-5Mt}}{5} \dots\right]$$

Since e is of a negative exponential order and M is always positive,

$$e^{-Mt} \gg \frac{e^{-3Mt}}{3} \gg \frac{e^{-5Mt}}{5}$$

Therefore a suitable approximation can be made by neglecting all terms after e^{-Mt}, or

$$E_i = E + \frac{2RT}{F} e^{-Mt} \tag{12}$$

Equation 12 is identical to the relation $y = k - Ae^{-ct}$. No comparison in constants can be made since the time measurements for the experimental curve involved a lapse of 5 min between zero and the time of the first potential reading.

It is a well-known fact that the equilibrium potential of the U_6,U_4 system assumes different values according to the intensity of light that illuminates the system. This light effect is referred to as the Becquerel effect. Heal attributes the phenomenon to the conversion of uranyl ions to an active form under the influence of light, which thereby have acquired augmented oxidative properties. As a result the velocity of formation of UO_2^+ is increased, since the reaction consists essentially of the oxidation of U_4 by U_6. This in turn alters the velocity constant of the formation reaction of UO_2^+. The over-all effect is an increase in the equilibrium concentration of UO_2^+, which will result in a new formation equilibrium constant K. Equation 12 will still apply to these conditions if the measurement of potential is started prior to the attainment of equilibrium by the formation reaction (i.e., when $t = 0$, $[U_5] = 0$). The final equilibrium potential, which is a function of K, can thus be shown to be related to light intensity.

The time-potential equation derived by Heal is slightly different (although of an exponential type) from that given above, since the initial conditions employed by Heal in his investigations were different from ours. Heal permitted the U_6,U_4 system to attain equilibrium in the dark or in low illumination before measurements were initiated, so that a small amount of U_5 existed in solution at $t = 0$. The solution was then subjected to an illumination, and the familiar exponential curve was obtained. After the U_6 and U_4 are mixed in the dark (or diffused light) and the potential is recorded immediately, the current increases exponentially to a steady value as described by Eq. 12 given above. Once the steady value is attained and the solution is illuminated, the potential increases once more to another steady state in a slightly different manner as described by Heal. This procedure may be repeated for different light intensities, and in each case the behavior is described by the following relation derived by Heal:

$$t = \frac{1.151}{kC_{\text{max}}} \log \left\{ \frac{\left[\text{antilog}\,\frac{2E_0}{0.059} + \text{antilog}\,\frac{E + E_0}{0.059} - \text{antilog}\,\frac{E_0}{0.059} - \text{antilog}\,\frac{E}{0.059}\right]}{\left[\text{antilog}\,\frac{2E_0}{0.059} - \text{antilog}\,\frac{E + E_0}{0.059} + \text{antilog}\,\frac{E_0}{0.059} - \text{antilog}\,\frac{E}{0.059}\right]} \right\} \qquad (13)$$

where k is the velocity constant of the disproportionation reaction, C_{max} is the concentration of UO_2^+ when the potential has become steady, E_0 is the steady potential difference under illumination, and E is the observed potential at time t.

BIBLIOGRAPHY

1. H. G. Heal, Rept. MC-95 (Oct. 20, 1944).
*2. E. Baur, Z. physik. Chem., 63: 689 (1908).
*3. N. Titlestad, Z. physik. Chem., 72: 264 (1910).
*4. H. Schiller, Z. physik. Chem., 80: 656 (1912).
*5. G. Truempler, Z. physik. Chem., 90: 385 (1915).
*6. T. Swensson, Arkiv Kemi, Mineral., Geol., 7: No. 19 (1919).
7. J. C. Ghosh, Z. physik. Chem., B3: 419 (1929).

*Cited by Heal.

APPENDIX B

By G. H. Tishkoff

1. URANIUM IN NONCOMPLEXING MEDIA

The polarography of uranium in a noncomplexing medium throws considerable light upon the individual electron-transferring systems that comprise the U_6,U_4 system. The polarographic method affords a means whereby these systems may be studied under a number of varying conditions, so that reversibility as well as other properties may be investigated. Since the cathodic U_6,U_5 wave and the anodic U_4,U_5 wave are of prime importance in regard to the over-all U_6,U_4 potential, it is advisable to sum up the properties of these systems as found polarographically.

1.1 Cathodic Wave. 1. The wave occurs invariably at +0.066 volt based on the normal hydrogen electrode in a noncomplexing medium.

2. The half-wave potential is independent of acidity.

3. Analysis of the wave indicates a 1-electron change.

4. The reaction is a reversible one and consists of $UO_2^{++} + e = UO_2^+$.

5. The effect of dismutation of UO_2^+ on this wave is negligible in solutions of pH greater than 1.

1.2 Anodic Wave. 1. The wave occurs in acid solutions of pH greater than 2 at about +0.216 volt based on hydrogen.

2. The half-wave potential shows a decided dependence upon acid concentration.

3. Analysis of the wave indicates a 1-electron change.

4. Evidence has been obtained for the existence of UO^{++}.

5. The reaction is a reversible one and consists of

$$UO^{++} - e + H_2O = UO_2^+ + 2H^+$$

The ionic pairs associated with the individual polarographic waves have been identified, and suitable mechanisms of the electrode reactions have been proposed. The conclusions concerning the mechanism are in no way incongruous with those pertaining to the U_6,U_4 system at a smooth platinum electrode.

It has also been established that the kinetics of the reactions that occur at the mercury surface are in many respects unlike those involved in the mechanism of the U_6,U_4 redox potential at platinum. For example, a small role is played by the dismutation reaction. The equilibrium values of the platinum potentials and the half-wave potentials, once established, may be treated in an empirical manner as in the following pages. Emphasis will be placed upon the relation of polarographic half-wave potentials to the platinum-electrode potentials.

The polarographic wave for the cathodic process as represented by the reaction $UO_2^{++} + e = UO_2^+$ is described by

$$E_{\mathrm{d.e.}} = \underset{6,5}{(E_0)_s^*} - 0.06 \log \frac{C^\circ_{UO_2^+}}{C^\circ_{UO_2^{++}}} \qquad (1)$$

where $E_{\mathrm{d.e.}}$ is the applied voltage, $\underset{6,5}{(E_0)_s}$ is the standard redox potential for the U_6,U_5 system in noncomplexing medium, and C° is the concentration of uranium at the mercury surface. By application of diffusion theory to Eq. 1, it follows that

$$E_{\mathrm{d.e.}} = \underset{6,5}{(E_0)_s} - 0.06 \log \frac{k_{UO_2^{++}}}{k_{UO_2^+}} - 0.06 \log \frac{i}{i_d - i} \qquad (2)$$

where k is the Ilkovic constant.

Since the only variable for a given experiment is $i/i_d - i$, the constant terms are combined, and the half-wave potential for the U_6,U_5 system is written as

$$E_{\frac{1}{2}} = \underset{6,5}{(E_0)_s} - 0.06 \log \frac{k_{UO_2^{++}}}{k_{UO_2^+}} \qquad (3)$$

Since $k_{UO_2^+} \approx k_{UO_2^{++}}$

$$E_{\frac{1}{2}} = \underset{6,5}{(E_0)_s} \qquad (4)$$

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[*] The standard potential for a noncomplexing medium will be denoted as $(E_0)_s$; for a complexing medium the designation will be $(E_0)_c$.

Therefore, the half-wave potential of the cathodic wave is very nearly equal to the redox potential of the U_6,U_5 system. $\underset{6,5}{(E_0)_s}$ is equal to +0.066 volt based on hydrogen.

In a similar manner, the polarographic wave for the anodic process as given by the reaction $UO^{++} - e + H_2O = UO_2^+ + 2H^+$ is described by

$$E_{d.e.} = \underset{4,5}{(E_0)_s} - 0.06 \log \frac{(C^\circ_{UO^{++}})}{(C^\circ_{UO_2^+})(C^\circ_{H^+})^2} \tag{5}$$

and

$$E_{d.e.} = \underset{4,5}{(E_0)_s} - 0.06 \log \frac{k_{UO_2^+}}{k_{UO^{++}}} + 0.06 \log \frac{i}{i_d - i} - 0.12\ \text{pH} \tag{6}$$

It follows that the half-wave potential is given by the equation

$$E_{\frac{1}{2}} = \underset{4,5}{(E_0)_s} - 0.12\ \text{pH} \tag{7}$$

since $k_{UO_2^+} \approx k_{UO^{++}}$. From the behavior of $E_{\frac{1}{2}}$ with pH, $\underset{4,5}{(E_0)_s}$ may be evaluated by means of Eq. 7. An average value of +0.572 volt based on hydrogen was found.

Thus, the polarograph provides a method whereby the individual redox potential of the 1-electron systems that comprise the U_6,U_4 system may be evaluated and treated in a quantitative manner. It can be shown that an underlying relationship that may be used to deal quantitatively with the experimental data is as follows:

$$\underset{6,4}{E_0} = \frac{\underset{6,5}{E_0} + \underset{4,5}{E_0}}{2}$$

This is derived as follows: Let F be the Faraday, and ΔF the free-energy change for the reaction. Then the following relationships hold:

$$U_6 + e = U_5 \qquad \underset{6,5}{\Delta F} = \underset{6,5}{E_0} F$$

$$U_5 + e = U_4 \qquad \underset{6,5}{\Delta F} = \underset{4,5}{E_0} F$$

$$U_6 + 2e = U_4 \qquad \underset{6,4}{\Delta F} = \underset{6,4}{E_0} 2F$$

The summation of the free-energy changes of the reduction of U_6 and U_5 is equal to the free-energy change of the reduction of U_6 to U_4.

$$\underset{6,4}{\Delta F} = \underset{6,5}{\Delta F} + \underset{4,5}{\Delta F} = (\underset{6,5}{E_0} + \underset{4,5}{E_0})F = \underset{6,4}{E_0} 2F$$

or

$$\underset{6,4}{E_0} = \frac{\underset{6,5}{E_0} + \underset{4,5}{E_0}}{2} \tag{8}$$

Equation 8 applies either to complexing or noncomplexing media. By substitution of $\underset{6,5}{(E_0)_s}$ and $\underset{4,5}{(E_0)_s}$ into Eq. 8, a value of +0.324 volt vs. the normal hydrogen electrode (N.H.E.) is obtained for the E_0 of the U_6,U_4 system in a noncomplexing medium. Taylor and Smith[1] reported a value of +0.328 volt by direct determination with the platinum electrode. This value of the redox potential for the U_6,U_4 system in a weakly acid medium is widely different from +0.407 volt reported by Khlopin and Gurevich[2] in strongly acid media and is further evidence to support the existence of the UO^{++} ion in our investigations.

The hypothesis has been presented in Appendix A that the U_6,U_4 potential at a smooth platinum electrode is established by the U_6,U_5 pair alone, and that the dismutation reaction sets up equilibriums between the U_6,U_4, U_6,U_5, and U_5,U_4 pairs. The dismutation reaction is the rate-determining reaction at the platinum electrode in setting up the final equilibrium potential. It is therefore convenient at times to express the U_6,U_4 potential as a function of the U_6,U_5 potential plus the dismutation constant. From the information procured by means of the polarograph and by the use of the relations given above, this hypothesis has been rigorously supported and placed on a firm mathematical foundation.

It is possible to write two different formation-disproportionation reactions: one reaction involving hydroxyl ions, the other hydrogen ions.

(i) $$UO_2^{++} + UO^{++} + 2OH^- = 2UO_2^+ + H_2O$$

(ii) $$UO_2^{++} + UO^{++} + H_2O = 2UO_2^+ + 2H^+$$

The formation constant of Eq. (i) is

$$(K_{OH})_s = \frac{[UO_2^+]^2}{[UO_2^{++}]\,[UO^{++}]\,[OH^-]^2} \tag{9}$$

The formation constant is denoted as $(K_{OH})_s$ to indicate the reaction in which hydroxyl ions are involved and in a noncomplexing solution. In like manner, the acid-formation constant is given as

$$(K_H)_s = \frac{[H^+]^2\,[UO_2^+]^2}{[UO_2^{++}]\,[UO^{++}]} \tag{9a}$$

The disproportionation constant is the reciprocal of the formation constant. Solving Eq. 9 for (UO_2^+), the following equation is obtained:

$$[UO_2^+] = \sqrt{(K_{OH})_s \cdot [UO_2^{++}]\,[UO^{++}]\,[OH^-]^2}$$

Since $K_w = [H]\,[OH]$,

$$[UO_2^+] = K\,\frac{[UO_2^{++}]^{\frac{1}{2}}\,[UO^{++}]^{\frac{1}{2}}}{[H^+]} \tag{9b}$$

where

$$K = \sqrt{(K_{OH})_s} \cdot K_w = \sqrt{(K_H)_s} \qquad \text{(from Eq. 9a)}$$

The reduction of U_6 to U_5 at the bright platinum electrode is given by the Nernst equation as follows:

$$E = \underset{6,5}{E_0} - 0.06 \log \frac{[UO_2^+]}{[UO_2^{++}]} \tag{10}$$

Likewise the oxidation of U_4 to U_5 at platinum is given as

$$E = \underset{4,5}{E_0} - 0.06 \log \frac{[UO^{++}]}{[UO_2^+]\,[H^+]^2} \tag{10a}$$

Substituting the expression for $[UO_2^+]$ from Eq. 9b in Eq. 10 gives

$$E = \underset{6,5}{E_0} - 0.06 \log K\,\frac{[UO_2^{++}]^{\frac{1}{2}}\,[UO^{++}]^{\frac{1}{2}}}{[H^+]\,[UO^{++}]}$$

and

$$E = \underset{6,5}{E_0} - \frac{0.06}{2} \log \frac{[UO^{++}]}{[UO_2^{++}]\,[H^+]^2} - 0.06 \log K \tag{11}$$

Substituting the expression for $[UO_2^+]$ from Eq. 9b in Eq. 10a gives

$$E = \underset{4,5}{E_0} - 0.06 \log \frac{[UO^{++}]\,[H^+]}{K\,[UO_2^{++}]^{\frac{1}{2}}\,[UO^{++}]^{\frac{1}{2}}\,[H^+]^2}$$

and

$$E = \underset{4,5}{E_0} - \frac{0.06}{2} \log \frac{[UO^{++}]}{[UO_2^{++}]\,[H^+]^2} + 0.06 \log K \qquad (11a)$$

Equations 11 and 11a now describe the U_6,U_4 system in a noncomplexing medium except that $\underset{6,4}{E_0}$ is replaced by either $\underset{6,5}{E_0}$ or $\underset{4,5}{E_0}$ and a function of K. Therefore the right-hand members of Eqs. 11 and 11a may be equated with the following result:

$$\begin{aligned} \underset{4,5}{(E_0)_s} + 0.06 \log K &= \underset{6,5}{(E_0)_s} - 0.06 \log K \\ \underset{6,5}{(E_0)_s} - \underset{4,5}{(E_0)_s} &= 0.12 \log K \end{aligned} \qquad (12)$$

Equation 12 is similar to that derived by Michaelis[3] for the formation constant of an intermediate radical. It is apparent from Eq. 12 that the value of K will determine whether $\underset{6,5}{(E_0)}$ is less or greater than $\underset{4,5}{(E_0)_s}$. In other words, the fact that the half-wave potential of the anodic wave is more positive than the half-wave potential of the cathodic wave can be attributed to the particular value of K.

K can also be evaluated from the standard potential of the U_6,U_4 system by a modification of Eq. 12 through the use of Eq. 8.

$$\underset{6,4}{(E_0)_s} = \underset{6,5}{(E_0)_s} - 0.06 \log K \qquad (12a)$$

$$\underset{4,5}{(E_0)_s} = \underset{6,4}{(E_0)_s} - 0.06 \log K \qquad (12b)$$

K may be evaluated numerically by appropriate substitutions in Eqs. 12, 12a, or 12b. It was found to be 5×10^{-5}. The basic formation constant $(K_{OH})_s$ calculated from K was 25×10^{18}. The acid-formation constant is equivalent to the square of K or 25×10^{-10}. The values used for $\underset{6,5}{(E_0)_s}$ and $\underset{4,5}{(E_0)_s}$ are those obtained from polarographic data.

Inasmuch as the platinum-electrode studies were conducted in a complexing medium and the polarographic method as described thus far in a noncomplexing medium, the conclusions concerning the U_6,U_5 and U_4,U_5 systems as well as their redox potentials, when applied to the U_6,U_4 system at platinum, must necessarily be made with restraint. However, when the polarographic method is applied directly to a complexing medium, considerable information may be procured relevant to the U_6,U_4 system. These results will be discussed in the following section.

2. URANIUM IN COMPLEXING MEDIA

The complex physiological composition and high pH of the body fluids favor the existence of uranium complexes, and it was therefore essential to extend the polarographic investigations to media that contain complex anions. Even though the studies on uranium in acid media gave abundant and pertinent results, these results could not be extrapolated to physiological conditions, as was the purpose of this investigation.

In this section the experiments and results that were obtained in media containing simple organic anions will be discussed. This information obtained by the polarographic method, together with that procured from the platinum-electrode studies, has made it possible to arrive at some interesting conclusions on the probable state of uranium under physiological conditions.

It has been found that the anions of the simple organic acids, such as acetate and bicarbonate, were highly suitable for studies on uranium complexes, since they render themselves adaptable to the polarographic method. That is to say, the polarographic waves are well defined, reproducible, and occur in a potential range suitable for polarographic work.

It is advisable to sum up the properties of the cathodic U_6,U_5 wave and the anodic U_4,U_5 wave found in acetate media in a manner similar to that which has been done for a noncomplexing medium.

2.1 Cathodic Wave. 1. The wave occurs invariably at approximately +0.004 volt based on hydrogen in a sodium acetate-acetic acid buffer.

2. The half-wave potential is independent of acidity but shows a decided dependence upon the acetate-ion concentration.

3. Analysis of the wave indicates a 1-electron change.

4. The reaction is a reversible one and consists of the reduction of the uranyl acetate complex to the pentavalent state.

5. The effect of dismutation of UO_2^+ on the cathodic wave is negligible in acetate buffer media.

2.2 Anodic Wave. 1. The wave occurs in acetate media at about +0.150 volt based on hydrogen.

2. The half-wave potential shows a decided dependence upon acid and acetate-ion concentration.

3. Analysis of the wave indicates a 1-electron change.

4. The reaction is a reversible one and consists of the oxidation of uranous acetate complex to the pentavalent state.

Equilibrium values of the platinum potentials and the half-wave potentials in a complexing medium will be treated in a quantitative manner as given below. Emphasis will again be placed upon the relation of polarographic half-wave potentials to the platinum-electrode potentials.

Let the cathodic reduction of U_6 at the mercury surface be expressed by the following equation:

$$N_bH^+ + [(UO_2^{++})\,(Ac^-)_p]^{+(2-p)} + e = \left[UO_{\left(2-\frac{N_b}{2}\right)}\,(Ac^-)_q\right]^{+(1+N_b-q)} + (p-q)Ac^- + \frac{N_b}{2}\,H_2O$$

where N_b is the number of hydrogen ions involved. This representation does not propose any definite uranium acetate structure. Then the polarographic wave is described by

$$E_{d.e.} = \underset{6,5}{(E_0)_c} - 0.06 \log \frac{C^\circ_{U_5}\left(C^\circ_{Ac}\right)^{p-q}}{C^\circ_{U_6}\left(C^\circ_{H^+}\right)^{N_b}} \tag{13}$$

where E_0 is the thermodynamic redox potential, and C° is the concentration of the ion at the mercury surface. The concentration is assumed to be equal to activity. C_{U_6} and C_{U_5} represent the concentration of the uranium complexes. If a suitable buffer is present, i.e., acetate, the concentration of hydrogen ions is constant throughout the solution and the dropping-mercury potential may be written as

$$E_{d.e.} = \underset{6,5}{(E_0)_c} - 0.06 \log \frac{C^\circ_{U_5}\left(C^\circ_{Ac}\right)^{p-q}}{C^\circ_{U_6}} - 0.06\,(N_b)\,\text{pH} \tag{13a}$$

Likewise, if an excess of acetate ion is present from the buffer system,

$$E_{d.e.} = \underset{6,5}{(E_0)_c} - 0.06 \log \frac{C^\circ_{U_5}}{C^\circ_{U_6}} - 0.06\,(N_b)\,\mathrm{pH} - 0.06\,(p-q) \log C_{Ac} \qquad (13b)$$

where C_{Ac} represents the excess acetate concentration present from the buffer. By application of diffusion theory to Eq. 13b, the following expression can be derived to describe the U_6,U_5 system at the dropping-mercury electrode in a complexing medium:

$$E_{d.e.} = \underset{6,5}{(E_0)_c} - 0.06 \log \frac{k_{U_6}}{k_{U_5}} - 0.06\,(N_b)\,\mathrm{pH} - 0.06 \log \frac{i}{i_d - i} - 0.06\,(p-q) \log C_{Ac} \qquad (13c)$$

where k is the Ilkovic constant.

Since the only variable for a given experiment is $i/(i_d - i)$, the constant terms are combined, and the half-wave potential for the U_6,U_5 system is written as

$$E_{\frac{1}{2}} = \underset{6,5}{(E_0)_c} - 0.06 \log \frac{k_{U_6}}{k_{U_5}} - 0.06\,(N_b)\,\mathrm{pH} - 0.06\,(p-q) \log C_{Ac} \qquad (14)$$

The anodic reaction at the mercury surface is

$$\left[UO_{\left(2-\frac{N_c}{2}\right)}(Ac^-)_r\right]^{+(N_c-r)} + (q-r)Ac^- + \frac{N_c}{2}H_2O - e = \left[UO_2(Ac^-)_q\right]^{+(1+N_b-q)} + N_cH^+$$

In a manner similar to that already outlined for the treatment of the cathodic wave, there is obtained for the anodic reaction the following equation:

$$E_{d.e.} = \underset{4,5}{(E_0)_c} - 0.06 \log \frac{C^\circ_{U_4}\left(C^\circ_{Ac}\right)^{q-r}}{C^\circ_{U_5}\left(C^\circ_{H^+}\right)^{N_c}} \qquad (15)$$

$$E_{\text{d.e.}} = (E_0)_{\underset{4,5}{c}} - 0.06 \log \frac{k_{U_5}}{k_{U_4}} + 0.06 \log \frac{i}{i_d - i} - 0.06\,(N_c)\,\text{pH} - 0.06\,(q-r) \log C_{Ac} \tag{15a}$$

and

$$E_{\frac{1}{2}} = (E_0)_{\underset{4,5}{c}} - 0.06 \log \frac{k_{U_5}}{k_{U_4}} - 0.06\,(N_c)\,\text{pH} - 0.06\,(q-r) \log C_{Ac} \tag{16}$$

The number of hydrogen ions involved in both the cathodic and anodic reactions can be found by maintaining the acetate concentration constant. Since all other terms are constant, $E_{\frac{1}{2}}$ can be plotted as a function of pH alone. From the plot, N_b and N_c can be evaluated. With these conditions the half-wave potential of the cathodic wave was found to be independent of pH ($N_b = 0$). N_c for the anodic wave was found to be 2 from the experimental slope of 0.12 volt per pH unit.

A series of experiments was conducted in which pH was constant and acetate concentration varied. From Eqs. 14 and 16 it is seen that, if $E_{\frac{1}{2}}$ is plotted against log C_{Ac}, $p - q$ and $q - r$ may be determined from the slope.

The results are shown in Fig. 1.40. When this method was applied to the cathodic wave of a 0.0001M uranyl acetate solution at pH 5.0, a straight line was obtained, and a value of 2 found for $p-q$ over the range of acetate 0.4M to 0.005M (see curve A, Fig. 1.40). The same method applied to the anodic wave gave two linear slopes at pH 5.0 (curve B, Fig. 1.40). The acetate concentration at which the change in slope occurred was about 0.05M. Above this acetate concentration $q - r = -1$. Below this concentration $q - r = -2$. If the ratio of acetate to uranium is decreased by increasing the uranium concentration to 0.001M, then this break in slope occurs at a higher acetate concentration. This is probably to be expected from dilution and mass-action effects.

Since q is involved in three slopes in Fig. 1.40, only certain discrete values can be substituted for p, q, and r without introducing a discrepancy. An additional restriction is imposed by the fact that p, q, and r cannot become larger as the acetate concentration decreases. It can be shown to follow that the only values for p, q, and r are 3, 1, and 2, respectively, above 0.05M acetate, and 2, 0, and 2, respectively, below 0.05M acetate at a uranium concentration of 0.0001M.

A correlation between the polarographic half-wave potentials and the platinum equilibrium potentials can be made in the following manner: The reaction at the platinum electrode of the U_6,U_4 system in the presence of acetate may be written as

$$2H^+ + [(UO_2^{++})(Ac^-)_p]^{+(2-p)} + 2e = [(UO^{++})(Ac^-)_r]^{+(2-r)}$$

$$+ H_2O^+(p - r)Ac^-$$

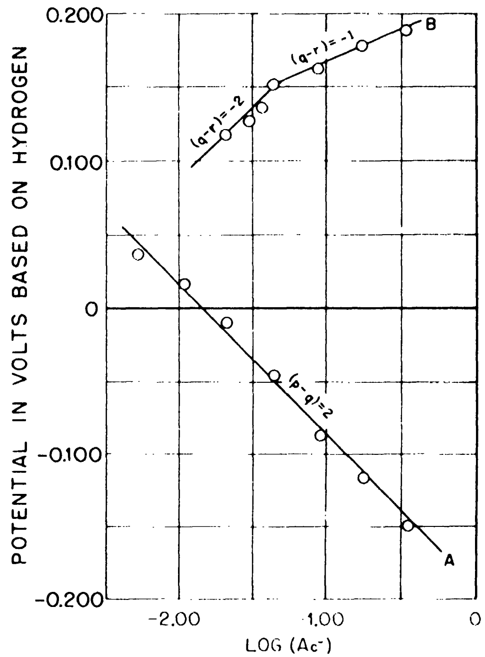

Fig. 1.40—Half-wave potentials of the U_6,U_5 and the U_4,U_5 systems vs. logarithm of the acetate-ion concentration.

The variation of potential as described by the Nernst equation is

$$E = (E_0)_{\substack{c\\6,4}} - 0.03 \log \frac{[UO(Ac^-)^r]\,[Ac^-]^{p-r}}{[UO_2(Ac^-)_p]\,[H^+]^2} \qquad (17)$$

It follows that for an equimolar U_6,U_4 system

$$E_0'' = (E_0)_c + 0.06 \log [H^+] - 0.03\,(p - r) \log [Ac^-] \qquad (18)$$

where E_0 is the standard electrode potential. Equation 17 also would describe the potential behavior at the dropping-mercury electrode if such a reaction were to take place there. By applying the diffusion theory to Eq. 17, the following expression can be derived to describe the U_6,U_4 system at the dropping mercury electrode:

$$E_{d.e.} = \underset{6,4}{(E_0)_c} - 0.03 \log \frac{k_{U_6}}{k_{U_4}} - 0.03 \log \frac{i}{i_d - i} + 0.06 \log [H^+] - 0.03\,(p - r) \log [Ac^-] \qquad (19)$$

$E_{\frac{1}{2}}$ can be evaluated by the following equation:

$$\underset{6,4}{E_{\frac{1}{2}}} = \underset{6,4}{(E_0)_c} - 0.03 \log \frac{k_{U_6}}{k_{U_4}} + 0.06 \log [H^+] - 0.03\,(p - r) \log [Ac^-] \qquad (20)$$

Therefore, $\underset{6,4}{E_0}$ may be expressed either by Eqs. 18 or 20.

It follows that

$$\underset{6,4}{E_0''} = \underset{6,5}{E_{\frac{1}{2}}} + 0.03 \log \frac{k_{U_6}}{k_{U_4}} \qquad (21)$$

But $k_{U_6} \approx k_{U_4}$. Therefore, as a close approximation,

$$\underset{6,4}{E_0''} = \underset{6,4}{E_{\frac{1}{2}}} \qquad (21a)$$

It has been established that the relationship

$$\underset{6,4}{E_0} = \frac{\left(\underset{6,5}{E_0} + \underset{4,5}{E_0}\right)}{2}$$

applies to a noncomplexing medium. By means of this relation, the platinum-electrode potentials can be related to data obtained through use of the dropping-mercury electrode. It is possible to show that the same relationship applies when a complexing medium consisting of acetate buffer is employed. When Eqs. 14 and 16 are added and divided by two, the following equation is obtained:

$$\frac{\underset{6,5}{E_{\frac{1}{2}}} + \underset{4,5}{E_{\frac{1}{2}}}}{2} = \frac{\underset{6,5}{(E_0)_c} + \underset{4,5}{(E_0)_c}}{2} - 0.03 \log \frac{k_{U_6}}{k_{U_4}} - 0.03\ (p - r) \log C_{Ac}$$
$$- 0.03\ (N_b + N_c)\ pH$$

Since $k_{U_6} \approx k_{U_4}$ and $N_b + N_c = 2$, the following is a close approximation:

$$\frac{\underset{6,5}{E_{\frac{1}{2}}} + \underset{4,5}{E_{\frac{1}{2}}}}{2} = \frac{\underset{6,5}{(E_0)_c} + \underset{4,5}{(E_0)_c}}{2} - 0.03\ (p - r) \log C_{Ac} - 0.06\ pH \qquad (22)$$

From Eq. 8

$$\frac{\underset{6,5}{E_{\frac{1}{2}}} + \underset{4,5}{E_{\frac{1}{2}}}}{2} = \underset{6,4}{(E_0)_c} - 0.03\ (p - r) \log C_{Ac} - 0.06\ pH \qquad (22a)$$

On comparison with Eqs. 18 and 21a,

$$\frac{\underset{6,5}{E_{\frac{1}{2}}} + \underset{4,5}{E_{\frac{1}{2}}}}{2} = \underset{6,4}{E_{\frac{1}{2}}} = \underset{6,4}{E_0''} \qquad (23)$$

It is recalled that it was necessary in working with the bright platinum electrode to establish optimum conditions for rapid equilibration of the U_6,U_4 system. The optimal uranium concentration was approximately 0.004M in total uranium. Equation 23 has been verified experimentally as shown by Fig. 1.41. Curves A and B represent the effect of pH upon the cathodic and anodic half-wave potentials. Curve C represents the equilibrium potential of an equimolar U_6,U_4 system (total uranium = 0.004M) under optimal conditions plotted against pH. If Eq. 23 holds, then the sum of the half-wave potentials at any given pH divided by two should equal the equilibrium potential. Curve D shows excellent agreement in the pH range 2.5 to 5.5.

The value of $\underset{6,4}{(E_0)_c}$ found directly by Eq. 8 was +0.346 volt based on hydrogen under the conditions $p = 2$, $q = 0$, and $r = 2$. When $p = 3$, $q = 1$, and $r = 2$, $\underset{4,5}{(E_0)_c}$ assumes another value, and $\underset{6,4}{(E_0)_c}$ was found to be +0.302 volt based on hydrogen.

When the dropping-mercury electrode is employed as an indicator electrode, a value of +0.313 volt vs. N.H.E. was obtained for $\underset{6,4}{(E_0)_c}$

under the conditions p = 2, q = 0, and r = 2. This value is somewhat different from the value of +0.346 volt vs. N.H.E., which was obtained in the manner described above. The discrepancy can be attributed partly to the fact that optimal concentration conditions were not in effect with the dropping-mercury electrode.

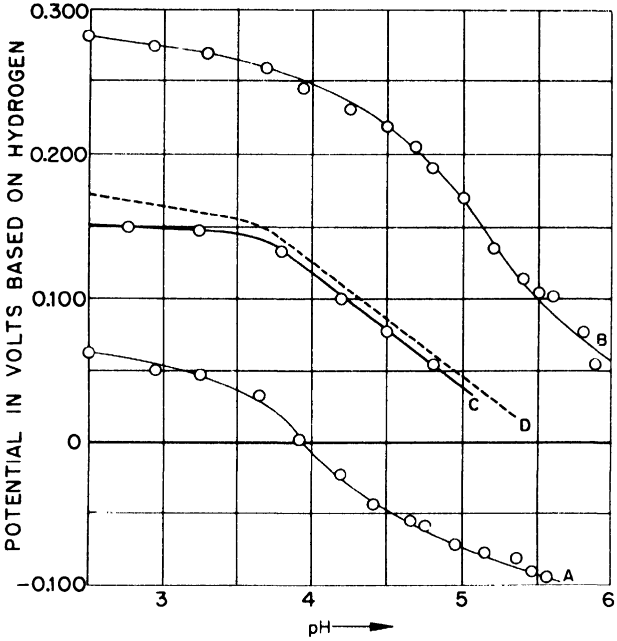

Fig. 1.41—Curves showing changes in half-wave potentials of the U_6,U_5; U_4,U_5; and U_6,U_4 systems in constant-strength acetate buffer with change in pH; also showing potential of the U_6,U_4 system at the platinum electrode under identical conditions. Curve A, $E_{\frac{1}{2}\,4,5}$; curve B, $E_{\frac{1}{2}\,5,4}$; curve C, $E_{\frac{1}{2}\,6,4}$; curve D, $E''_{0\,6,4}$ at platinum electrode.

Briefly, the way in which the polarograph is used as an indicator electrode is as follows: E_0 is determined from the intersection of the "current-voltage" curve and the 0-current line for a solution containing equimolar reductant and oxidant. Or the redox potential may be obtained in another manner by repeating the current-voltage curve at another sensitivity. The intersection of the two curves is identical with that obtained by intersection of the 0-current line and either of these curves.

From the data that have been accumulated on the behavior of the cathodic and anodic waves in acetate buffer, it has been possible, in a manner similar to that outlined above, to determine the acid and basic formation constants of $UO_2(Ac)_q$. However, because of the tendency of sodium acetate to complex with uranium, it is necessary to modify the dismutation reactions given in Eqs. 8a and 8b in Sec. 1 of this appendix in the following manner:

$$\text{(a)}\quad UO_2(Ac)_p + UO(Ac)_r + 2OH^- = 2UO_2(Ac)_q + (p - 2q + r)Ac^- + H_2O$$

$$\text{(b)}\quad UO_2(Ac)_p + UO(Ac)_r + H_2O = 2UO_2(Ac)_q + (p - 2q + r)Ac^- + H_2O$$

The basic and acid formation constants are expressed as

$$(K_{OH})_c = \frac{[UO_2(Ac)_q]^2\,[Ac^-]^{p-2q+r}}{[UO_2(Ac)_p]\,[UO(Ac)_r]\,[OH^-]^2} \tag{24}$$

$$(K_H)_c = \frac{[UO_2(Ac)_q]^2\,[Ac^-]^{p-2q+r}\,[H^+]}{[UO_2(Ac)_p]\,[UO(Ac)_r]} \tag{24a}$$

The $[UO_2(Ac)_q]$ is found either from Eqs. 24 or 24a as

$$[UO_2(Ac)_q] = (K)_c\,\frac{\sqrt{[UO_2(Ac)_p]\,[UO(Ac)_r]}}{[H^+]\,\sqrt{[Ac^-]^{p-2q+r}}} \tag{24b}$$

where $(K)_c = \sqrt{(K_{OH})_c} \times K_w = \sqrt{(K_H)_c}$.

The Nernst equation for the cathodic reduction of uranyl acetate is given as

$$E = \underset{6,5}{(E_0)_c} - 0.06 \log \frac{[UO_2(Ac)_q]\,[Ac^-]^{p-q}}{[UO_2(Ac)_p]} \tag{25}$$

Likewise, the oxidation of the uranous acetate complex to U_5 at platinum is given as

$$E = \underset{4,5}{(E_0)_c} - 0.06 \log \frac{[UO(Ac)_r]\,[Ac]^{p-r}}{[UO_2(Ac)_q]\,[H^+]^2} \tag{25a}$$

On substitution of the expression for $[UO_2(Ac)_q]$ given by Eq. 24b into Eq. 25, it can be seen that

$$\begin{aligned} E = \underset{6,5}{(E_0)_c} - 0.06 \log (K)_c - 0.03 \log \frac{[UO(Ac)_r]}{[UO_2(Ac)_p]} \\ + 0.03\,(p - 2q + r) \log [Ac^-] - 0.06\,\text{pH} \\ - 0.06\,(p - q) \log [Ac^-] \end{aligned} \tag{26}$$

Substitution of the expression for $[UO_2(Ac)_q]$ as given by Eq. 24b into Eq. 25a yields the following expression:

$$\begin{aligned} E = \underset{4,5}{(E_0)_c} + 0.06 \log (K)_c - 0.03 \log \frac{[UO(Ac)_r]}{[UO_2(Ac)_p]} \\ - 0.03\,(p - 2q + r) \log [Ac^-] - 0.06\,\text{pH} \\ - 0.06\,(q - r) \log [Ac^-] \end{aligned} \tag{26a}$$

Consider the conditions where $p = 2$, $q = 0$, and $r = 2$, i.e., below 0.05M acetate and with the uranium concentration equal to 0.0001M. These conditions will be designated by use of the subscript c2.

Under these conditions Eqs. 26 and 26a become

$$E = \underset{6,5}{(E_0)_{c2}} - 0.06 \log (K)_{c2} - 0.03 \log \frac{[UO(Ac)_r]}{[UO_2(Ac)_p]} - 0.06\,\text{pH}$$

$$E = \underset{4,5}{(E_0)_{c2}} + 0.06 \log (K)_{c2} - 0.03 \log \frac{[UO(Ac)_r]}{[UO_2(Ac)_p]} - 0.06\,\text{pH}$$

Since both relations describe the identical system, U_6,U_4, the right-hand members may be equated,

$$\begin{aligned} \underset{6,5}{(E_0)_{c2}} - 0.06 \log (K)_{c2} = \underset{4,5}{(E_0)_{c2}} + 0.06 \log (K)_{c2} \\ \underset{6,5}{(E_0)_{c2}} - \underset{4,5}{(E_0)_{c2}} = 0.12 \log (K)_{c2} \end{aligned} \tag{27}$$

Equation 27 is identical in form to Eq. 12 except that it applies to a complexing medium. It can also be shown by identical procedures as in Appendix B, Sec. 1, that

$$\underset{6,5}{(E_0)_{c2}} - \underset{6,4}{(E_0)_{c2}} = 0.06 \log (K)_{c2} \tag{27a}$$

and

$$\underset{6,4}{(E_0)_{c2}} - \underset{4,5}{(E_0)_{c2}} = 0.06 \log (K)_{c2} \tag{27b}$$

$(K)_{c2}$ was found from Eq. 27. By use of Eqs. 14 and 16 under known conditions, $\underset{6,5}{(E_0)_{c2}}$ was evaluated as -0.458 volt against the standard calomel electrode (S.C.E.) (-0.212 vs. N.H.E.) and $\underset{4,5}{(E_0)_{c2}}$ was found to be equal to $+0.669$ volt vs. S.C.E. ($+0.915$ vs. N.H.E.) with the polarograph. $(K)_{c2}$ was found to be equal to 4×10^{-10}. It follows that $(K_{OH})_{c2} = 16 \times 10^{8}$ and $(K_H)_{c2} = 16 \times 10^{-20}$.

Consider the conditions where $p = 3, q = 1$, and $r = 2$; i.e., the acetate concentration is greater than 0.05M and the uranium concentration is 0.0001M. These conditions will be designated by the subscript c3.

Under these conditions Eqs. 26 and 26a become,

$$E = \underset{6,5}{(E_0)_{c3}} - 0.06 \log (K)_{c3} - 0.03 \log \frac{[UO(Ac)_r]}{[UO_2(Ac)_p]}$$

$$+ 0.03\ (3) \log [Ac^-] - 0.06\ (2) \log [Ac^-] - 0.06\ \mathrm{pH}$$

$$E = \underset{4,5}{(E_0)_{c3}} + 0.06 \log (K)_{c3} - 0.03 \log \frac{[UO(Ac)_r]}{[UO_2(Ac)_p]}$$

$$- 0.03\ (3) \log [Ac^-] + 0.06\ (1) \log [Ac^-] - 0.06\ \mathrm{pH}$$

Equating the right-hand members of these relations, the following equation is obtained,

$$\underset{6,5}{(E_0)_{c3}} - 0.06 \log (K)_{c3} + 0.03\ (3) \log [Ac^-] - 0.12 \log [Ac^-] =$$

$$\underset{4,5}{(E_0)_{c3}} + 0.06 \log (K)_{c3} - 0.03\ (3) \log [Ac^-] + 0.06 \log [Ac^-]$$

Simplifying,

$$\underset{6,5}{(E_0)_{c3}} - \underset{4,5}{(E_0)_{c3}} = 0.12 \log (K)_{c3}$$

By use of Eqs. 14 and 16 as applied under known conditions, $\underset{6,5}{(E_0)_{c3}}$

was evaluated as −0.458 volt vs. S.C.E. (−0.212 volt vs. N.H.E.) and $(E_0)_{c3}$ as +0.57 volt vs. S.C.E. (+0.816 vs. N.H.E.) by means of the[4,5] polarograph. $(K)_{c3}$ was found to be equal to 2.7×10^{-9}. It follows that $(K_{OH})_{c3} = 7.3 \times 10^{10}$ and $(K_H)_{c3} = 7.3 \times 10^{-18}$.

3. CALCULATION, FROM POLAROGRAPHIC DATA, OF DISSOCIATION CONSTANTS OF URANIUM COMPLEXES OF VARIOUS VALENCE STATES

In this section a discussion will be given of the method whereby the polarograph may be used to determine the dissociation constants of uranium complexes for the various valence states of uranium. The examples studied are, as before, the acetate complexes of uranium as formed in a sodium acetate-acetic acid buffer.

The following equilibriums pertaining to the dissociation of U_6, U_5, and U_4 acetate complexes can be set up:

$$UO_2(Ac)_p \rightleftharpoons pAc^- + UO_2^{++} \; ; \; UO_2(Ac)_p = \frac{[UO_2^{++}]\,[Ac^-]^p}{K_6} \tag{28}$$

$$UO_2(Ac)_q \rightleftharpoons qAc^- + UO_2^{+} \; ; \; UO_2(Ac)_q = \frac{[UO_2^{+}]\,[Ac^-]^q}{K_5} \tag{29}$$

$$UO(Ac)_r \rightleftharpoons rAc^- + UO^{++} \; ; \; UO(Ac)_r = \frac{[UO^{++}]\,[Ac^-]^r}{K_4} \tag{30}$$

An expression for the acid and basic formation constants in acetate buffer in terms of K_6, K_5, and K_4 can be obtained by substitution of Eqs. 28, 29, and 30 into Eqs. 24 and 24a as given previously.

$$\begin{aligned}(K_{OH})_c &= \frac{K_6K_4}{K_5^2} \times \frac{[UO_2^{+}]^2}{[UO_2^{++}]\,[UO^{++}]\,[OH^-]^2}\\ (K_{OH})_c &= \frac{K_6K_4}{K_5^2} \times (K_{OH})_s\end{aligned} \tag{31}$$

$$\begin{aligned}(K_H)_c &= \frac{K_6K_4}{K_5^2} \times \frac{[UO_2^{+}]^2[H^+]^2}{[UO_2^{++}]\,[UO^{++}]}\\ (K_H)_c &= \frac{K_6K_4}{K_5^2} \times (K_H)_s\end{aligned} \tag{31a}$$

Under the conditions where $p = 2$, $q = 0$, and $r = 2$, K_6K_4 was found from Eqs. 31 and 31a to be 6.4×10^{-11}, since K_5 does not exist. However, when $p = 3$, $q = 1$, and $r = 2$, K_6K_4/K_5^2 was found to be 30×10^{-10}.

The displacement of the half-wave potentials of a definite uranium system in passing from a noncomplexing medium to a complexing acetate solution is related to the dissociation constants of the acetate complex by the following relation as given by Kolthoff and Lingane.[4]

$$\underset{U_{ox},U_{re}}{(E_{\frac{1}{2}})_c} - \underset{U_{ox},U_{re}}{(E_{\frac{1}{2}})_s} = 0.06 \log \frac{k_{U_{ox-ac}}}{k_{U_{re-ac}}} - 0.06\,(p - q) \log [Ac^-] \qquad (32)$$

where $(E_{\frac{1}{2}})_c$ and $(E_{\frac{1}{2}})_s$ are the half-wave potentials of the polarographic waves in acetate solution and noncomplexing solutions, respectively. $K_{U_{ox-ac}}$ and $K_{U_{re-ac}}$ represent the dissociation constants of the oxidized and reduced form of the system investigated into free ions.

From the system U_6,U_5, $\underset{6,5}{(E_0)_s} = -0.18$ volt vs. S.C.E. and $p - q = 2$ as determined from polarographic data. In the pH range 3.8 to 5.5 an average value of 2.2×10^{-5} was obtained for K_6/K_5. In a similar manner K_5/K_4 was found to be 4.3×10^5 when $q - r = -2$, and 1.1×10^4 when $q - r = -1$. A value of $+0.336$ volt vs. S.C.E. was employed for $(E_0)_S$ as found polarographically. It should be pointed out that, under the conditions $q = 0$, pentavalent uranium has zero acetates attached and consequently cannot be included in the relations given above. The value of 2.2×10^{-5} is the numerical value of the constant K_6. Likewise, 4.3×10^5 is the value of the constant $1/K_4$.

It can be shown that the standard electrode potentials of the U_6,U_4 system are related to the dissociation constants of the components in the following manner as given by Kolthoff and Lingane.[4]

$$\underset{6,4}{(E_0)_c} - \underset{6,4}{(E_0)_s} = 0.03 \log \frac{K_6}{K_4} \qquad (33)$$

where $\underset{6,4}{(E_0)_c}$ and $\underset{6,4}{(E_0)_s}$ are the standard electrode potentials in acetate and noncomplexing solutions, respectively. From Eq. 33, K_6/K_4 was found to be 5.41 when $p = 2$, $q = 0$, and $r = 2$.

The term K_6/K_4 can also be evaluated from the ratios K_6/K_5 and K_5/K_4. Under the same conditions,

$$\frac{K_6}{K_5} \times \frac{K_5}{K_4} = \frac{K_6}{K_4} = 2.2 \times 10^{-5} \times 4.3 \times 10^5 = 9.5$$

This value is of the same order of magnitude as the value 5.41, which was found by using Eq. 33. Under the conditions $p = 3$, $q = 1$, $r = 2$, K_6/K_4 was found to be 0.184 by Eq. 33. This result compares favorably with the value of 0.242 found from K_6/K_5 and K_5/K_4.

A value of 6.4×10^{-11} for the product K_6K_4 was reported as determined from the formation constants in noncomplexing and complexing solutions. Under the conditions where $q = 0$, K_5 no longer exists, and therefore the product K_6K_5 can be evaluated from the previously given equation. However, when $q = 1$, K_5 exists, and the product K_6K_4/K_5^2 must change in accordance with $(K_H)_{c3}$ in order to maintain a constant value of $(K_H)_s$ (see Eq. 31a). K_6K_4 and K_6K_4/K_5^2 may also be established from the values of K_6 and K_4, and the ratios K_6/K_5 and K_4/K_5 as found from Eq. 32.

(A) $p = 2$, $q = 0$, $r = 2$.

$$K_6K_4 = 2.2 \times 10^{-5} \times 2.4 \times 10^{-6} = 5.28 \times 10^{-11}$$

(B) $p = 3$, $q = 1$, $r = 2$.

$$\frac{K_6K_4}{K_5^2} = \frac{K_6}{K_5} \times \frac{K_4}{K_5} = 2.2 \times 10^{-5} \times 9.17 \times 10^{-5} = 20.2 \times 10^{-10}$$

These results compare favorably with those obtained above by use of Eqs. 31 and 31a. From the information thus obtained, the following dissociation constants can be written for the uranium acetate complexes. The designation K_{x-y} will be used to represent the dissocation

$$U(Ac)_x = (x-y)Ac^- + U(Ac)_y$$

Case A. $p = 2$, $q = 0$, $r = 2$.

Under these conditions K_5 does not exist.

$$K_4 = K_{2-0} = 2.4 \times 10^{-6}, \text{ and } K_6 = K_{2-0} = 2.2 \times 10^{-5}$$

If it is assumed that the acetate bonds attached to the uranium atom are uniform,[5] then $K_{2-1}/K_{1-0} = 4$. Consequently, K_{2-1} and K_{1-0} may be evaluated.

Case B. $p = 3$, $q = 1$, $r = 2$.

Since r is still equal to 2, $K_4 = 2.4 \times 10^{-6}$ as found in A. However, K_4/K_5 is now 9.2×10^{-5}. Therefore, K_5 is found to be $2.6 \times 10^{-2} = K_{1-0}$ for the dissociation,

$$UO_2(Ac^-)_1 = Ac^- + UO_2^+$$

From the ratio $K_6/K_5 = 2.2 \times 10^{-5}$, K_6 is evaluated as $5.7 \times 10^{-7} = K_{3-0}$, which is the equilibrium constant for the dissociation

$$UO_2(Ac^-)_3 = 3Ac^- + UO_2^{++}$$

$K_{3-2} = K_6$ can be obtained in the following manner:

$$K_{3-0} = K_{3-2} \times K_{2-0}$$

or

$$5.7 \times 10^{-7} = 2.2 \times 10^{-5} \times K_{3-2}$$

$$2.5 \times 10^{-2} = K_{3-2}$$

The dissociation of the uranium acetate complexes may be summarized as follows:

Hexavalent Uranium:

$UO_2(Ac)_3^- = 3Ac^- + UO_2^{++}$	$K_{3-0} = 5.7 \times 10^{-7}$
$UO_2(Ac)_2 = 2Ac^- + UO_2^{++}$	$K_{2-0} = 2.2 \times 10^{-5}$
$UO_2(Ac)_2 = Ac^- + UO_2(Ac)_1^+$	$K_{2-1} = 9.35 \times 10^{-3}$
$UO_2(Ac)_1^+ = Ac^- + UO_2^{++}$	$K_{1-0} = 2.34 \times 10^{-3}$
$UO_2(Ac)_3^- = Ac^- + UO_2(Ac)_2$	$K_{3-2} = 2.5 \times 10^{-2}$
$UO_2(Ac)_3^- = 2Ac^- + UO_2(Ac)_1^+$	$K_{3-1} = 2.43 \times 10^{-4}$

Pentavalent Uranium:

$UO_2(Ac)_1^+ = Ac + UO_2^+$	$K_{1-0} = 2.6 \times 10^{-2}$

Tetravalent Uranium:

$UO(Ac)_2 = 2Ac^- + UO^{++}$ $\quad K_{2-0} = 2.4 \times 10^{-6}$

$UO(Ac)_2 = Ac^- + UO(Ac)_1^+$ $\quad K_{2-1} = 3.08 \times 10^{-3}$

$UO(Ac)_1^+ = Ac^- + UO^{++}$ $\quad K_{1-0} = 0.77 \times 10^{-3}$

BIBLIOGRAPHY

1. J. K. Taylor and E. R. Smith, Nat. Bur. of Standards (U. S.), Rept. A1972 (Aug. 29, 1944).
2. V. G. Khlopin and A. M. Gurevich, Bull. acad. sci. U. R. S. S., Classe. sci. chim., 1943: 271-279 (1943).
3. L. Michaelis, Chem. Rev., 22: 437 (1938).
4. I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, 1941.
5. E. Q. Adams, J. Am. Chem. Soc., 38: 1503 (1916).

Chapter 2

ANALYTICAL METHODS

By John F. Flagg*

The problem of making chemical analyses for various substances that are important in the pharmacological and toxicological studies is one of considerable magnitude. The effects being studied are those produced by minute amounts of the various uranium or other compounds, in view of which the analytical problem is almost entirely one of semimicroanalysis or microanalysis. Further complications arise from the fact that the elements to be determined are generally found in biological samples and the like, the complexity of which requires a considerable amount of preliminary treatment prior to making what otherwise would be a straightforward analysis. Thus conventional methods have had to be adapted to the special type of problem encountered in these studies, and in some cases entirely new methods have been developed for some specific purpose. The subject of this chapter is the description of these analytical methods.

The two elements receiving most attention with regard to analysis have been uranium and fluorine, and Parts A and B of the chapter deal with analytical methods for them. In Part B, a special method for analyzing air samples is described.

In some cases analytical methods other than those to be described have been used, as for example in the determination of purity and composition of chemicals. As these methods have been adequately described elsewhere, their inclusion here does not seem justified.

In presenting the details of each method as used in the project laboratory, the aim is to supply sufficiently complete information to enable the method to be used directly under similar circumstances.

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*Work done by John F. Flagg, W. R. Bloor, William F. Neuman, G. H. Tishkoff, L. T. Steadman, Frank A. Smith, Ralph Lobene, Ann Tarbell, Marion J. Voss, Leo B. Cohenour, William Davis, Charles Horton, Carrol Weil, and Max Schlamowitz.

PART A. METHODS FOR DETERMINING URANIUM

1. COLORIMETRIC DETERMINATION OF URANIUM USING FERROCYANIDE*

The colorimetric determination of uranium with ferrocyanide depends on the formation of a red-brown color when a weakly acid solution of the uranyl ion is treated with potassium ferrocyanide solution. The method has been the subject of much investigation, and in the present modification is adapted to the determination of uranium in dust samples in amounts ranging upward from 80 μg.

The first step in the analysis of dust samples consists in the collection of the dust from air, either by means of filter paper, an impinger, or an electrostatic precipitator. Following this the uranium is converted to the hexavalent form by oxidation with nitric acid, after which the color is developed and measured in a suitable instrument.

1.1 Preparation of Sample. Filter-paper samples are prepared for analysis by ashing in porcelain crucibles over a free flame. Then the ash is transferred to a pyrex evaporating dish, the liquid is evaporated to dryness on a hot plate, and the residue is dissolved in nitric acid. Electrostatic precipitator samples are removed from the tube by refluxing with nitric acid. In all cases the dissolved sample must be freed from excess nitric acid by careful evaporation, avoiding the formation of insoluble basic salts by too much evaporation. Approximately 1 drop of solution should remain after the evaporation.

The analysis of dust samples may be made more rapid by transferring to a special filtering assembly the filter paper upon which the dust has collected. The filtering assembly consists of a sintered-glass filter of the same diameter as the filter paper, which may be fitted into a volumetric flask and to which suction may be applied. The filter paper containing the dust sample is placed on the sintered-glass filter. The dust is dissolved in a minimum amount of 1 per cent acetic acid and drawn through the filter directly into the volumetric flask.

This method is considerably more rapid than the ashing procedure and gives equally good recoveries.

1.2 Development of Color. The color may be developed in a volume of 10, 25, or 50 ml, depending on the amount of uranium in the sample. For 80 to 400 μg of uranium, the volume should be 10 ml; for larger amounts of uranium, the proportionally larger volume is used, although solutions no stronger than 3.0 mg in 50 ml should be analyzed.

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* Method developed by Leo Cohenour and William Davis.

The uranium salt is taken up in 5 per cent acetic acid, and 5 per cent potassium ferrocyanide solution is added. The solution is then made up to the appropriate volume. In Table 2.1 are given the proper amounts of reagents to be used.

Table 2.1 — Reagents for Colorimetric Determination of Uranium

Total volume to be used, ml	5 % ferrocyanide to add, ml	5 % acetic acid to add, ml
50	5.0	5.0
25	2.5	2.5
10	1.0	1.0

After making up to volume with distilled water, the solutions are allowed to stand for 30 min at room temperature, in the light, and a measure of the color intensity is determined in a photoelectric colorimeter such as the Klett-Summerson. A series of standards, prepared from known amounts of uranium in the manner described, is used to obtain a calibration curve by use of which unknown amounts of uranium may be determined. A convenient source of uranium for the standards is U_3O_8, which is easily obtained in a high state of purity and which is readily converted into uranyl nitrate.

1.3 Discussion. The pH of the solution in which the color is developed will be approximately 3.1, provided care is taken to evaporate the nitric acid. This may be regarded as an optimum pH.

Interfering ions are those which form insoluble ferrocyanides, such as copper, lead, iron, etc., and those anions which form complexes with the uranyl ion. Acetate is in the latter category; in the presence of large amounts of this ion the rate of color development is retarded, and low values are found. Nitrate, chloride, and fluoride ions, however, cause no difficulty. Presumably other organic anions, such as citrate, would interfere and possibly also sulfate in large amounts.

The colorimetric ferrocyanide method has the advantage of comparative simplicity, both in apparatus required and in manner of performance. The range of concentrations that can be analyzed is rather large, assuming that concentrated samples can be diluted if necessary. The reproducibility is the same as that normally expected in colorimetric methods, provided a uniform procedure is followed.

2. THE FLUOROPHOTOMETRIC DETERMINATION OF URANIUM*

The fluorophotometric method for the determination of minute amounts of uranium depends upon the fact that traces of the element, when fused with sodium fluoride, produce a strong fluorescence under the influence of ultraviolet light. As the intensity of the fluorescence is a function of the quantity of uranium present, it becomes possible to determine uranium quantitatively by measuring the intensity of the fluorescent light and comparing it with the intensity produced by known amounts of uranium treated in a similar manner.

The fluorophotometric method for determining minute amounts of uranium in biological materials was adapted to toxicological studies by Bloor and modified subsequently by Neuman. In its original form the method employed a visual determination of the intensity of fluorescence, while in the modified form a photoelectric measurement was substituted.

The method is adaptable to the determination of uranium in a wide variety of biological materials, after making certain preliminary treatments that may be required. It operates at a level of concentrations (parts per million or less) not easily reached by methods other than the spectrochemical method (see Sec. 4), and with an accuracy quite satisfactory for most biochemical studies.

Details of both variations of the fluorophotometric method will be given, as each is suited to a particular set of conditions.

2.1 Bloor's Method. (a) General. The material containing uranium, dissolved in an aliquot (0.1 ml) of concentrated nitric acid, is transferred to a small platinum dish and ashed in the Bunsen flame. Fifty milligrams of sodium fluoride is added and the whole fused, conditions being arranged so that the fused mass covers evenly the whole bottom of the dish and does not extend outside it. The fluorescent color produced by ultraviolet light is measured by extinction in a complementary-colored solution (methylene blue) in the cups of an ordinary Duboscq colorimeter, the depth of solution necessary to extinguish the fluorescent color being taken as a measure of the fluorescence and hence of the amount of uranium present. Fluorescence is proportional to the amount of uranium present. A calibration curve is constructed with known amounts of uranium, and the color values of the unknowns are read against this curve.

(b) Apparatus. Light Source. A General Electric BH 4 100-watt lamp (black glass) with parabolic reflector is used as the light source.

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* Method developed by W. R. Bloor and William F. Neuman.

The light is passed through two 5-mm thicknesses of Corning 9863 black glass (red-purple Corex) onto the sample in the small platinum dish, which is supported in a holder on the mirror assembly of the colorimeter, the angle of the mirror being adjusted to pass the light through the instrument. The light source and colorimeter are encased in a suitable box for the protection of the operator, and the whole is set up in a dark closet. Readings require partial dark adaptation, which may require several minutes.

Measuring Instrument. A modern-type Duboscq colorimeter such that scale readings can be made with a small flashlight is required. The cups contain methylene-blue solution, the strength of which depends on the strength of the fluorescence to be measured, but which in any event must be accurately known. For the range 0.04 to 0.4 μg of uranium, the strength should be 6.25×10^{-5}M. The cup on the right side of the colorimeter contains methylene-blue solution, about 25 mm deep; that on the left only a small amount, since it is turned up tight and is used only as control.

The holder for the platinum dishes containing the fluorescent material is made of pasteboard or metal to fit over the mirror and is suitably masked so as to show only the required central portion of the platinum vessels, which slip in behind the mask with a sliding fit.

Platinum Vessels. These are made of platinum foil, 1 by ¾ in., 0.1 mm thick, and the shallow cups are about ½ in. in diameter. The cup is made by spinning the platinum down into a mold made by a cork borer in a piece of thin pasteboard or photographic film, about 0.25 mm thick. The thickness of the platinum is a compromise between the need for sufficient thickness to heat easily and sufficient stiffness to stand manipulation.

(c) Preparation of Samples. Urine. This is tested as collected. A 0.1-ml sample is taken and a measurement made. If the result falls within the limits of the setup, another measurement is made, and if there is reasonable agreement an average is taken. If the urine contains too little uranium for accurate measurement, it is treated with concentrated nitric acid to about 10 per cent strength, evaporated to dryness, and then made to volume, as desired, by solution in water with a little nitric acid. If too strong, it is diluted with water as required. If the urine is to be kept, it should be made 10 per cent acid with concentrated nitric acid.

Feces. The fecal material is covered with strong nitric acid and evaporated to dryness in a hood. (Beware of foaming and loss.) The material is dissolved in 10 per cent nitric acid to a suitable volume and centrifuged. An aliquot is taken from the clear liquid. If it is too strong, the whole is diluted, mixed well, made up to volume, and centrifuged as before. If it is too weak, a larger aliquot of the clear fluid is taken, evaporated to dryness, and made up to a smaller volume.

Tissue. Approximately 1 g of tissue—kidney, liver, bone, intestine, etc.—is dissolved in 5 ml of concentrated nitric acid, evaporated to dryness, then made to suitable volume with 10 per cent nitric acid.

(d) Accuracy. For pure solutions containing quantities of 1 μg of uranium or more, the error may be as small as 5 per cent. For quantities below this level, the error is at least 10 per cent, possibly more. In the smaller concentrations the slight fluorescence of the sodium fluoride itself (caused mostly by reflected visible light) is a disturbing factor. It is fairly constant from day to day for freshly fused samples, but fluorescence seems to develop on standing, and a fresh blank must be prepared daily. This residual fluorescence may be due to contamination from the air if uranium material is or has been used near by.

(e) Checks. Recovery of known, added amounts of uranium from urine, feces, extracts, blood extracts, kidney extracts, etc., can be made within the limits of error of the method.

(f) Procedure. A 0.1-ml portion of the sample is pipeted into one of the platinum dishes and set on a square of asbestos board over a flame that is adjusted to give free evaporation without bumping or excessive foaming. The sample is evaporated to dryness; then the little vessel is transferred to a loop of wire (iron, nickel, or platinum) and heated in the open flame until the contents are completely ashed and free from dark specks. To it is added 50 mg of sodium fluoride, and the whole is fused. Heat at above the fusion point is maintained for about 5 sec (the fused material should flow over the surface of the dish two or three times), and the mixture is then cooled. This is done by raising the dish slowly out of the flame, keeping the fused mixture in motion by tilting the holder from side to side until the melt solidifies. The fused mixture should cover the cup in the platinum dish, should be evenly distributed over it, and should not extend outside the cup. If these conditions are not fulfilled, the mixture should be fused again until the distribution is right. Much depends on even thickness and distribution of the fusion.

The sample is then ready for reading and is transferred to the darkroom, and the ultraviolet light is started.

(g) The Readings. The sample in the dish is inserted into the right-hand opening on the holder, a similar blank sample of fused sodium fluoride is inserted in the opening on the opposite side, and the holder is positioned in the colorimeter. The left-hand cup is screwed up tightly, and adjustment of the right-hand cup is made until the brightness of the two sides of the field is the same, as is done in colorimetric work. A reading is taken, the sample removed from the holder, turned end for end, and a second reading taken. The average of the readings is taken as the final reading; its value is converted into micrograms of uranium by comparing with the calibration curve.

The calibration curve is made by taking 0.1-ml samples of dilutions of a uranyl nitrate solution of known concentration.

(h) Cleaning the Platinum Dishes. First the dishes are boiled with water for 2 min. The water is then replaced with fresh water containing a few drops of nitric acid, and the boiling is continued for 2 or 3 min. The dishes are then rinsed and are ready for reuse. Occasionally the entire treatment must be repeated to clean a dish. After cleaning, the dishes are re-formed if necessary.

(i) Effect of Tissue Minerals. In the amounts of material used in investigations using this method (the equivalent of about 25 μg of uranium per determination), the minerals of the tissue were found to have no significant effect on the results. However, it will be noted in the following section that when the amounts of uranium are much smaller, and the amounts of tissue much larger, the minerals do have an effect on the results, and the procedures must be revised accordingly to meet this difficulty.

2.2 Neuman's Method. As in Bloor's method, use is made of the fluorescence produced in a sodium fluoride bead that has been fused with a uranium-containing material. In this variation of the method, an apparatus for rapid measurement of fluorescent intensities is used, and certain special chemical techniques have been developed to isolate the uranium from interfering elements when the analysis of particularly complex materials was required.

This section, then, will be devoted to a discussion of the apparatus required for making rapid analyses on large numbers of samples, as well as to the chemical procedures that must precede such measurements.

(a) Construction of Fluorophotometer. Light Sources. Several types of commercial ultraviolet sources have been investigated, including the General Electric BH 4, Mineralight Model V 41, and mercury-arc lamps. All these sources activate the sodium fluoride-uranium flux to a bright yellow-green fluorescence. In one of the photometers constructed a Conti-Glo Flood Lamp (Continental Lithograph Co., General Electric Bulb EH 4) was employed. This lamp will give satisfactory service when equipped with a constant-voltage transformer (Sola, Model 30808). In the absence of voltage control, variation in line voltage will cause variations in lamp output. In a second model of the fluorophotometer, a General Electric AH 4 bulb was used. This bulb is made of clear glass; hence it was necessary to interpose Corning filters 9863 and 5874 between the lamp and the sample.

Sample Holder. The technique of fusing the sample with sodium fluoride in platinum is used. The cup is made from foil 0.005 in. thick, 7/8 by 3/4 in., and has a circular indentation 7/16 in. in diameter and approximately 1/16 in. deep at the center.

The platinum foil is held in position by means of a brass holder. The bright platinum surface around the flux is covered by a blackened brass mask. The brass bar is equipped to accommodate five foils and has a spring-stop mechanism to facilitate centering of the samples.

The Photometer. For the measurement of extremely small quantities of uranium, a barrier-type photocell may prove to be too insensitive, even when a sensitive galvanometer is used. As amplification of the photocurrent is not possible, a vacuum phototube with amplifier unit must be used.

A photometer, such as Model 512 manufactured by the Photovolt Corporation, is very satisfactory from the point of view of stability, sensitivity, and ease of operation. A battery-operated amplifier, designed especially for use in the fluorophotometric measurements, may also be used. The circuit is shown in Fig. 2.1.

Optical System. The instrument must be designed so as to place the light source as near as possible to the sample. The light may be mounted at a 45-deg angle to the sample, and the emitted beam measured at 90 deg, or the light may be mounted at a 30-deg angle, thus permitting more compact design. It is necessary to interpose Corning filters 3484 and 9780 between sample and phototube.

Construction. General details of construction are shown in Figs. 2.2 to 2.9. Parts I to XI, except Part II, are of 28-gauge sheet metal; Part II is of 20-gauge sheet metal. The sample holder and base plate are constructed of brass. Copper tubing, 1/4 in. in outside diameter, is attached to the water chambers and extended 7 in. to the rear. Parts VIII and IX are attached to Part IV.

(b) Procedure. A 0.1-ml aliquot of the sample to be analyzed is pipeted into a platinum cup. The cup is placed on an asbestos board and evaporated to dryness on an electric hot plate. When dry, the cup is heated at high heat and allowed to char (if organic material is present). The asbestos board and cup are then removed and allowed to cool. Then, approximately 80 mg of sodium fluoride (Baker & Adamson, Reagent Grade) is added to the cup by means of a scoop. The sample is then fused twice with a Méker burner and read in the fluorophotometer.

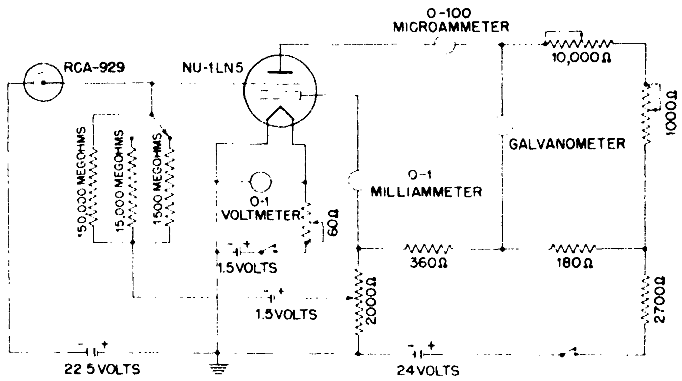

Fig. 2.1 -Electrometer circuit.

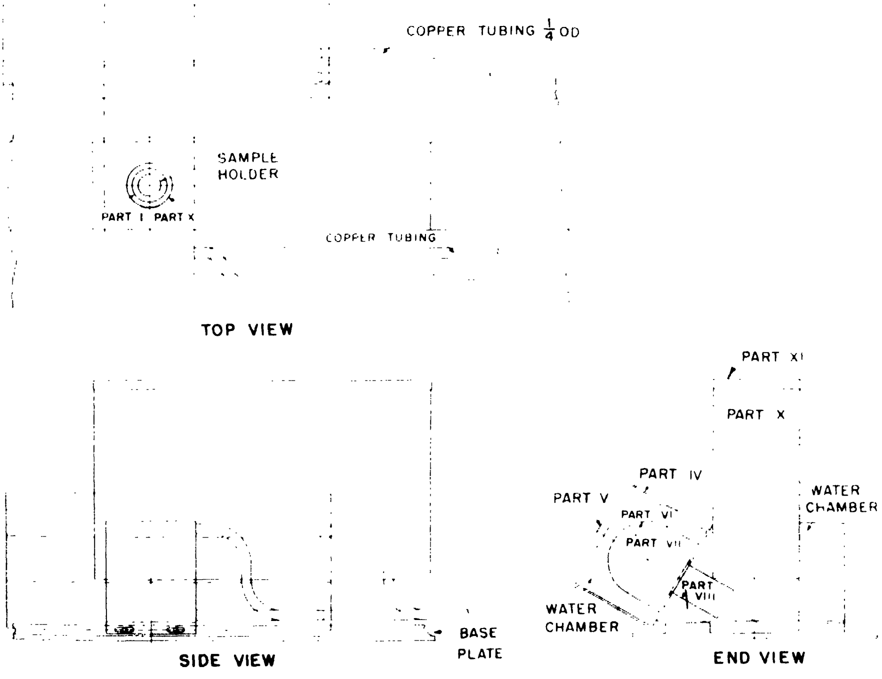

Fig. 2.2—Fluorophotometric attachment, Model V (see Figs. 2.3 to 2.9). Parts I-XI, except Part II, are of 28-gauge sheet metal. Part II is of 20-gauge sheet metal. Attach ¼-in. O.D. copper tubing to water chambers and extend 7 in. to the rear. Sample holder and base plate are of brass. Parts VIII and IX are attached to Part IV.

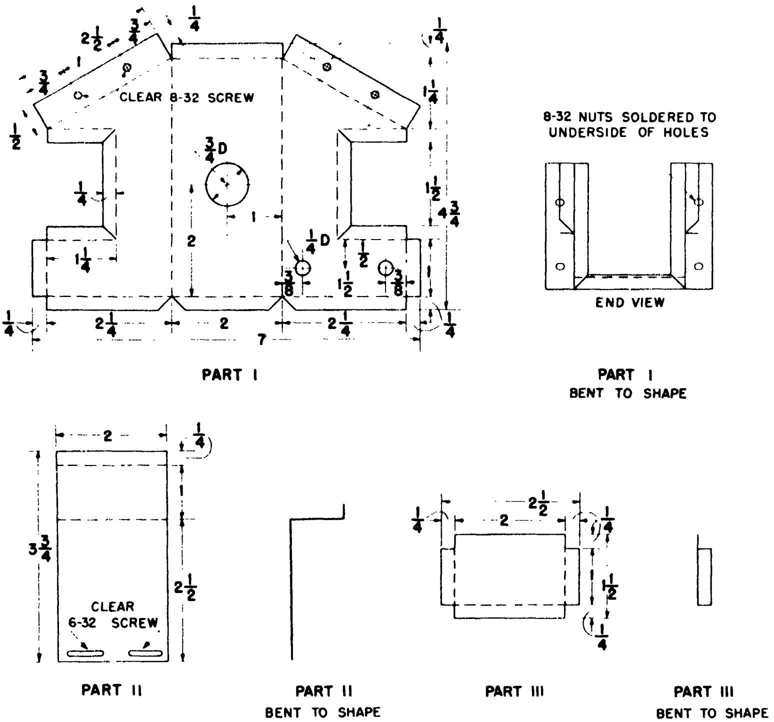

Fig. 2.3 — Fluorophotometric attachment (Cont.).

The quantity of sodium fluoride used in the fusion is not critical, variations in the amount of fluoride employed having relatively small effect on the fluorescence produced by a known amount of uranium. Care should be taken to ensure as uniform a fusion temperature as possible in order to obtain reproducible readings.

Before making the fluorescence readings, the ultraviolet lamp is allowed to warm up for at least ½ hr. A blank sample is read first, setting the galvanometer reading arbitrarily at some value (10 is convenient), which is subtracted from all subsequent readings. It is good practice to analyze all samples in quadruplicate, taking an average of the four readings. A convenient set of readings would consist, then, of four each for blank and sample, and one or more sets of four readings for standards. On pure solutions containing from 2 to about 0.2 μg of uranium per milliliter, an accuracy of 5 per cent or better might be expected.

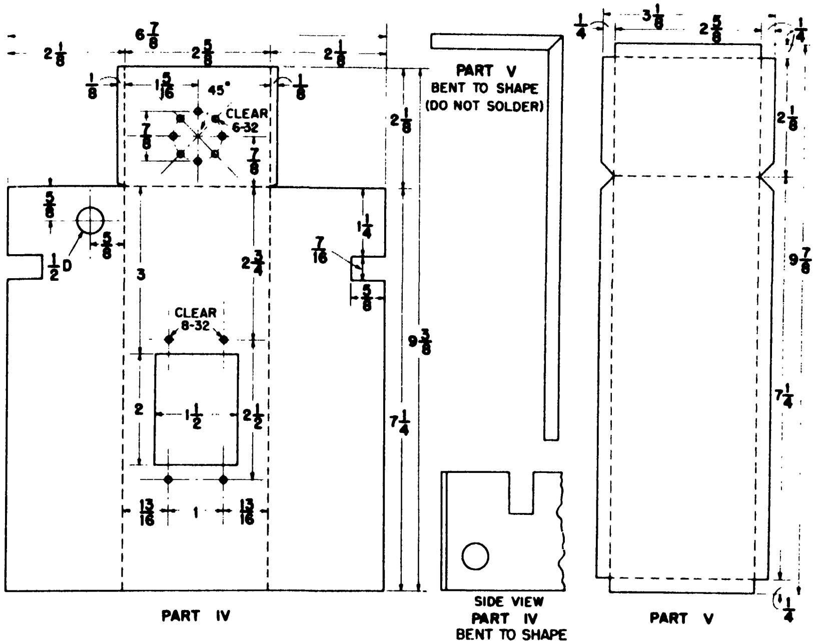

Fig. 2.4 — Fluorophotometric attachment (Cont.).

In order to analyze extremely small quantities of uranium, it is necessary to exercise a rigorous control over operating conditions. No dry uranium compounds may be permitted in the analytical room. All glassware (flasks, pipets, etc.) should be left overnight in cleaning solution and rinsed thoroughly in nitric acid and distilled water immediately before use. The platinum cups should be cleaned as previously described (Sec. 2.1h). Immediately before the sample is to be analyzed, the cups are again boiled with dilute nitric acid and rinsed several times with distilled water. Extreme care must be exercised during the period in which samples are pipeted, dried, and fused.

(c) Sources of Error in the Direct Fluorophotometric Determination of Uranium. As the first step in determining the uranium content of biological materials is the removal of organic matter, the ashing procedure is of utmost importance. Careful study has revealed that only from platinum crucibles can the recovery of uranium, following ashing, be regarded as quantitative. In the case of porcelain crucibles

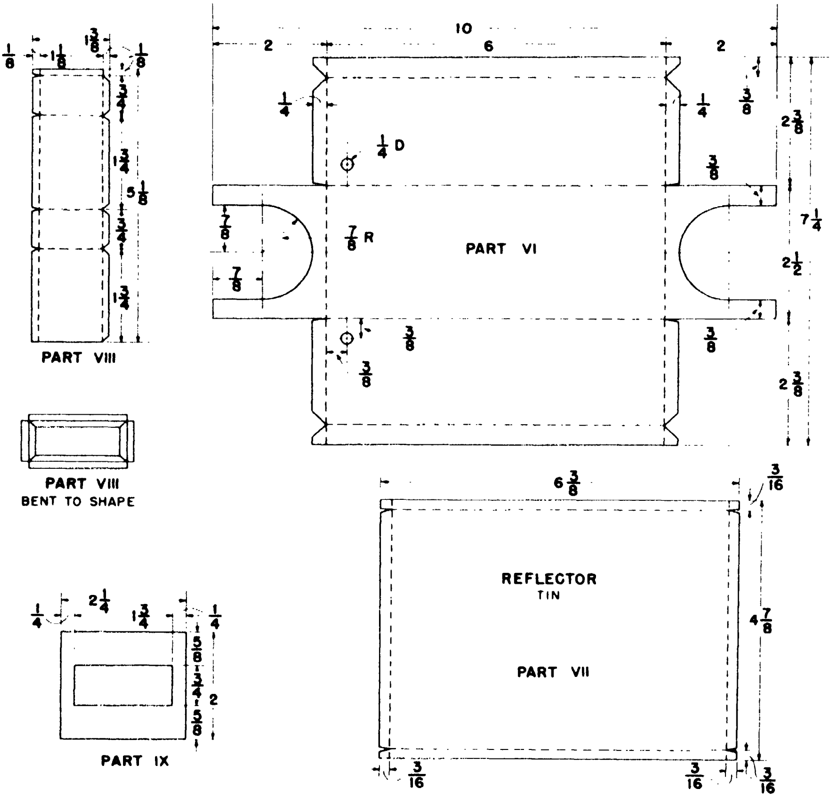

Fig. 2.5 — Fluorophotometric attachment (Cont.).

there may be an appreciable retention of uranium, possibly as a result of baking into the glaze of the crucible.

To prevent creeping of the molten ash inside the platinum crucibles, this ashing procedure should be followed: The sample is placed in the cold muffle furnace, which is then gradually heated to 400 to 450°C. The furnace is left at this temperature overnight, then the temperature is raised to 750°C for 1 to 2 hr, after which the sample is allowed to cool. This procedure will generally give a white or pale-yellow ash, readily soluble in 2N hydrochloric acid.

Another possible source of error arises from the presence of elements other than uranium in the sodium fluoride-uranium flux. Bone salt and iron both cause inhibition of the uranium fluorescence; other inorganic materials may behave in a similar manner. In view of this

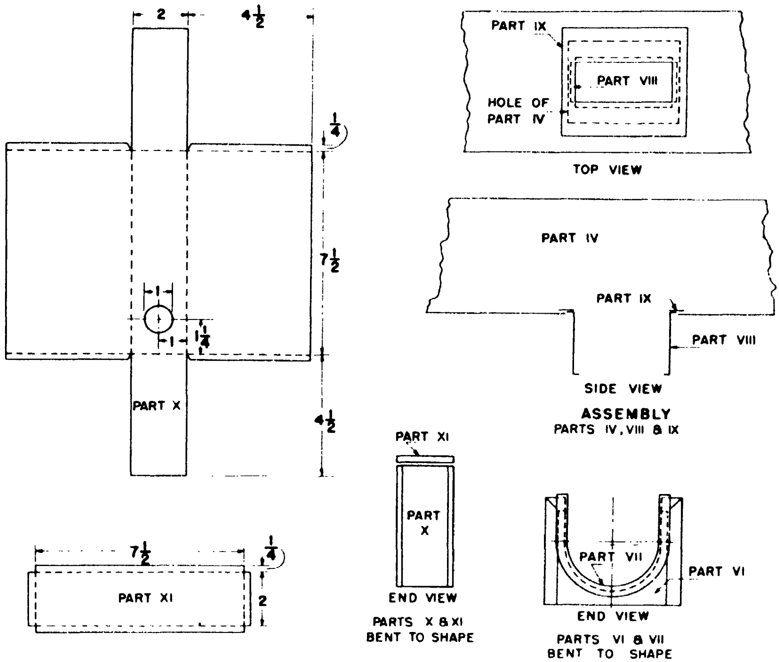

Fig. 2.6 — Fluorophotometric attachment (Cont.).

fact, it should be stated that the method as outlined is best suited for the analysis of relatively pure substances, in which the nature and extent of impurities, if any, are known. In all other cases preliminary isolation of the uranium should be carried out, in the manner to be described in the following section.

(d) Isolation of Uranium from Biological Materials. To be satisfactory for isolation of uranium from biological material, a method must be accurate, reproducible, and highly sensitive, as the amount of uranium sought will often be no more than a few micrograms or less in concentrations of a few parts in 10 million of fresh tissue. Furthermore, the uranium should be separated from the accompanying elements to the extent that they are no longer present in sufficient quantities to interfere in the fluorophotometric measurements.

Separation methods that would seem to fail to meet these requirements include direct precipitation with alkali, electroplating, adsorption, and precipitation of impurities rather than the uranium. In all cases incomplete recovery, or nonreproducible results, caused these methods to be discarded.

It has, however, been possible to make a more or less specific precipitation of uranium in amounts found in biological materials using egg albumin. This substance (either the commercial preparation or crystalline egg albumin) combines with the uranyl ion at pH of 4.5 to 5, in an acetate buffer, to form a complex that is extremely insoluble. The separation of uranium from bone salt, for example, is sufficiently quantitative to permit fluorophotometric analysis at concentrations of from 1 to 300 μg of uranium per gram of fresh bone. Interference with the precipitation is caused by citrate ions, which presumably form complexes with uranium and prevent its precipitation.

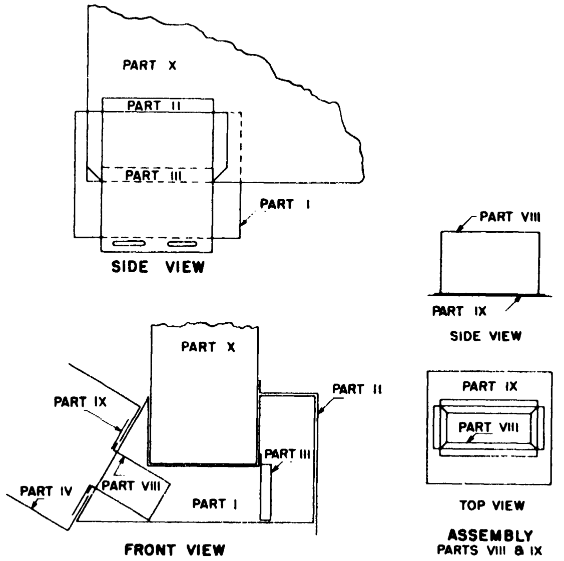

Fig. 2.7 — Fluorophotometric attachment (Cont.).

The volume of solution in which precipitation occurs is not critical, although for good recoveries of microgram quantities the concentration of albumin should be in excess of 2.5 mg/ml.

A source of error arises from the presence of iron, which is also precipitated in the protein-isolation process. Hence, when analyzing tissues such as blood, liver, and spleen, a preliminary separation of the iron must be made before the protein-isolation procedure.

(e) Procedure. The following procedure may be adopted for the routine analysis of biological specimens (except blood, liver, spleen).

The sample to be analyzed is placed in a platinum crucible and put in the muffle furnace overnight at 300°C. The temperature is raised to 700°C for 3 hr and the sample then removed. The ash is dissolved in a minimum of 2N hydrochloric acid with the aid of heat, and an aliquot containing no more than 100 mg of ash is transferred to a 50-ml centrifuge tube that is calibrated at 10 and 30 ml. Two drops of bromcresol green indicator are added, and the solution neutralized to pH 4.5 (green color) with sodium hydroxide. Three milliliters of 1N sodium acetate buffer (pH 5.0) and a solution of protein preparation (100 mg) are added. The solution is diluted to 30 ml. After thorough mixing, the tube is immersed in a hot-water bath at 80°C for 45 min. The coagulum is separated by centrifugation, washed once with 30 ml of fresh 0.1N sodium acetate buffer (pH 4.5), and again centrifuged. The washed precipitate is dissolved in concentrated nitric acid and made up to a volume of 10 ml.

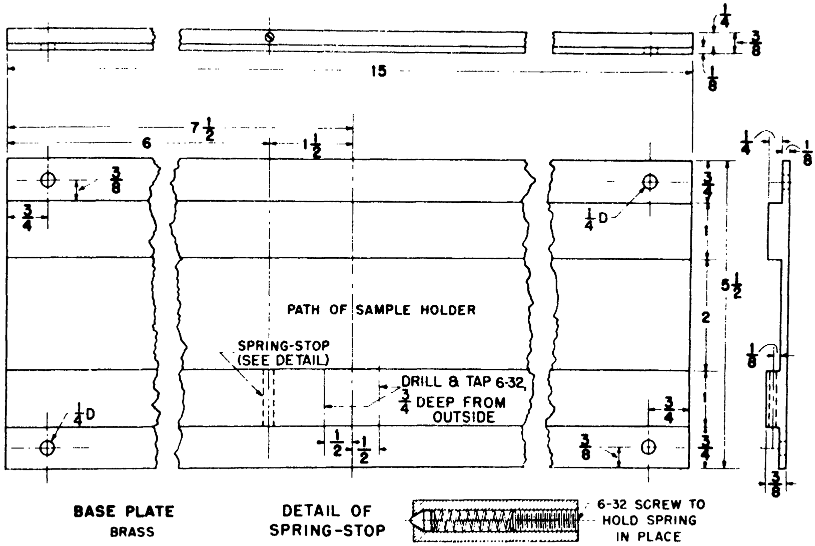

Fig. 2.8—Fluorophotometric attachment (Cont.).

One-tenth-milliliter aliquots are taken for fluorophotometric analysis. Blank determinations are carried through the procedure with each set of samples as a check against contamination of reagents.

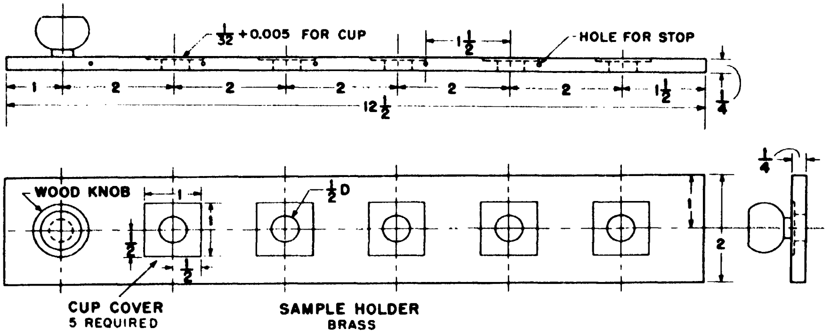

Fig. 2.9 — Fluorophotometric attachment (Cont.).

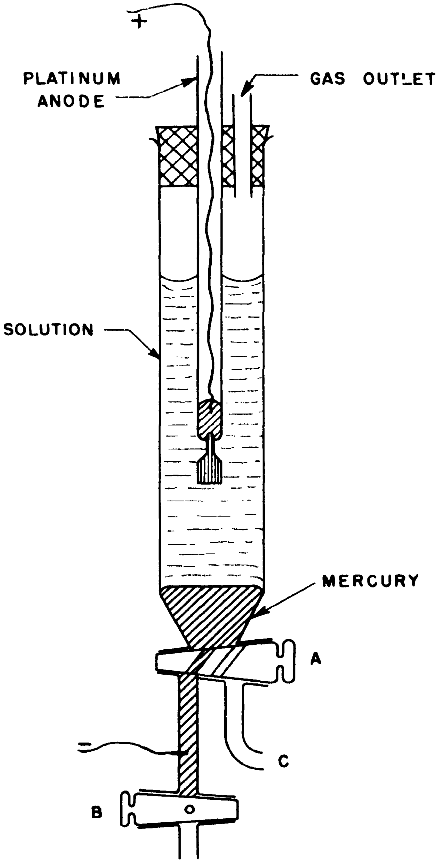

Fig. 2.10 — Mercury-cathode electrolysis cell.

(f) Removal of Iron. Iron may be removed by electrolysis into a mercury cathode, in a cell such as is shown in Fig. 2.10. The tissue ash, obtained as before, is taken up in 2N hydrochloric acid, and sufficient perchloric acid is added to make the concentration 3 per cent. The solution is placed in the cell and electrolyzed for 1 hr, using an applied potential of 3 volts d-c. Stopcock B is then opened and the mercury drawn into the capillary of stopcock A, which is then closed to interrupt the current. The iron-free solution is collected at outlet C. The solution is then washed into a centrifuge tube for subsequent protein isolation and fluorophotometric analysis.

Table 2.2 — Over-all Summary of Analytical Results

	Pure solutions	Tissues
No. of samples	16.0	61.0
Concentration range, ppm	0.01 - 10	0.5 - 1,000
Mean recovery, %	92.0	92.3
Standard deviation, %	7.0	7.0
Range in recovery, %	77 - 102	70 - 105

(g) Accuracy. The over-all accuracy of the protein isolation procedure and fluorophotometric analysis, employing electrolysis where necessary, is summarized by the data presented in Table 2.2. The results on pure solutions of uranium are given for comparison. Among the tissues tested have been samples of brain, liver, lung, kidney, spleen, intestine, muscle, gonads, urine, feces, and representative bones.

3. POLAROGRAPHIC METHOD FOR DETERMINING URANIUM*

The polarographic method for the determination of uranium depends on the fact that the uranyl ion is reduced at the dropping-mercury electrode, and that the diffusion current produced is proportional to the concentration of the uranyl ion in solution. For further details on the behavior of uranium at the dropping-mercury electrode, reference should be made to Chap. 1.

The method has been adapted to the determination of hexavalent uranium in various substances, particularly as traces in tetravalent uranium compounds. In such cases advantage is taken of the fact that the uranyl salts are soluble in hydrochloric acid and so may be removed from the bulk of the sample. The solution thus obtained is then analyzed polarographically, a particular advantage being that any tetravalent uranium compounds in solution will have no effect on the results.

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* Method developed by G. H. Tishkoff.

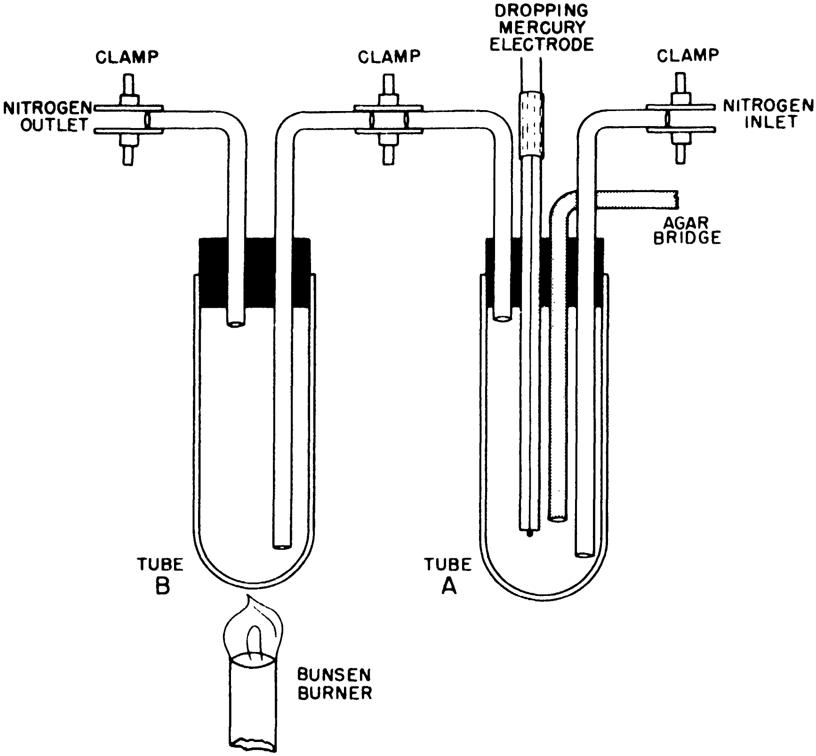

Fig. 2.11 — Cell for polarographic analysis.

The polarographic analyses are made in some form of apparatus employing electrolysis with a dropping-mercury electrode. Numerous modifications are on the market, including the Sargent-Heyrovsky polarograph, the Fisher Elecdropode, and the Leeds and Northrup Electrochemograph. All have in common the property of allowing current-voltage curves (or polarograms) to be made.

Hexavalent uranium is reduced at the dropping-mercury electrode at a potential of about −0.20 volt with respect to the saturated-calomel electrode. The height of the wave obtained is a direct function of the uranium concentration; if the wave heights for a number of known uranium solutions have been previously measured, then the concentration of an unknown is easily determined.

The apparatus used for the analysis of uranium tetrafluoride for hexavalent uranium is shown in Fig. 2.11. The apparatus is set up so that the nitrogen outlet from the dropping-mercury cell is inserted into another cell in which a second sample is being prepared for analysis.

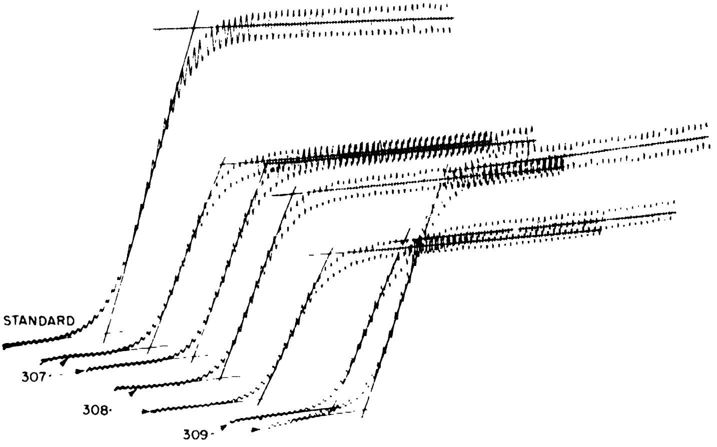

Fig. 2.12 — Polarograms obtained in analysis of "green salt" for hexavalent uranium.

The dropping-mercury cell consists of a round-bottom pyrex tube, 2.8 cm in diameter and 8 cm long. A saturated potassium chloride-calomel electrode is connected to the dropping-electrode cell by means of a saturated potassium chloride-agar bridge. The mercury capillary is about 10 cm in length.

Ten milliliters of a standard solution of 0.001M uranyl nitrate in 0.1N hydrochloric acid is placed in the dropping-mercury cell A, and pure nitrogen passed through for 15 min. During this time 10 ml of 0.1N hydrochloric acid is pipeted into sample tube B connected to the nitrogen outlet of tube A and containing the sample to be analyzed. The contents of the tube is heated to boiling, while maintaining a constant flow of nitrogen through both tubes until tube B has cooled to room temperature. Since no nitrogen can pass through the dropping-mercury cell when the polarogram is made, tube B is sealed from the atmosphere with a suitable clamp while the polarogram is being run.

After the polarogram is made, the standard solution is removed, and the electrodes and agar bridge are washed down with distilled water. The tube containing the sample is connected to the mercury electrode, and the procedure repeated with another sample. The total time during which nitrogen is passed through each individual solution is 30 min. Usually two runs are made on each sample of material to be analyzed, using a new sample for each analysis.

Some typical results are shown in Fig. 2.12.

The method is particularly valuable for the analysis of hexavalent uranium in uranium tetrafluoride ("green salt"). No lengthy procedures are required for separating the hexavalent from the tetravalent uranium; the method is direct and much more rapid than conventional volumetric procedures in which total uranium, then tetravalent uranium, must be determined in order to find by difference the amount of hexavalent uranium in the sample.

The average deviation of duplicate results on a single sample is less than ±3 per cent. This also represents the accuracy of the method.

4. THE SPECTROCHEMICAL DETERMINATION OF URANIUM IN BIOLOGICAL MATERIALS*

The spectrochemical method for the determination of uranium in biological materials involves the excitation of the uranium atoms in a sample by means of a d-c arc, recording the spectrum produced, and, from the measurement of the intensities of the uranium lines thus recorded, determining the quantity of uranium present in the sample. In certain types of analysis the uranium may be determined directly following an ashing procedure; in other cases it is necessary to concentrate the uranium from a comparatively large sample before making the measurements. The first process will be referred to as the "direct method," while the latter will be designated as the "ether concentration method."

A description of the spectrochemical method must treat the two principal steps involved in carrying out the method: (1) preparation of the sample, which is essentially a chemical process, and (2) spectrographic techniques, including production, recording, and analysis of the spectrum. A description of the various steps in both of the methods mentioned will be given.

4.1 Direct Method. (a) Preparation of Material. Fresh tissues or fluids are dried and then ashed overnight in porcelain crucibles in a muffle furnace at 500°C. The residues are moistened with 1 : 1 HNO_3 and reheated in the furnace to 400°C. The ash from small samples or from samples believed to contain large amounts of uranium is taken up in 1 : 1 HNO_3, and the aliquot chosen volumetrically such that about 0.2 ml of solution, which also contains the internal standard, is added to the electrode and dried therein. Residues from large samples are ground in a mortar, thoroughly mixed, and weighed. Samples of 10 or 30 mg are placed in the crater of the electrode to which has been added the internal standard. An enhancing agent is also used. Fluids that are to be extracted with ether are more conveniently prepared by acid digestion, which will be discussed in Sec. 4.2.

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* Method developed by L. T. Steadman.

Electrodes. Electrodes used are regular-grade National Carbon Company spectroscopic carbons, 0.25 in. in diameter. The lower electrode containing the sample and negative in polarity is cut 1.5 in. long and is provided with a crater 0.25 in. deep and 0.16 in. in diameter. The electrodes are purified by heating at about 100°C in a boiling flask with a reflux condenser for 4 days in 5 per cent HNO_3, continuing 4 days in 5 per cent HCl, and for 4 days in triple-distilled water, all solutions being changed daily. Sample electrodes are prearced for 30 sec.

Excitation of Sample. The sample in the carbon arc is burned for 2.5 min with an arc current of 12 amp or more and an applied potential of 130 volts d-c. Some improvement in speed of burning and sensitivity is obtained by supplying extra oxygen to the arc by means of a chimney about the sample electrode. This consists of an asbestos-cement-lined brass cylinder, 1.5 in. in diameter, closed at the bottom and open at the top, which is about 0.25 in. above the tip of the crater. A rectangular notch permits the light to illuminate the slit of the spectrograph. Regular oxygen is introduced at the bottom at a pressure of about 2 cm of H_2O.

Illumination of Slit. The two electrodes are arranged in a line about 15 deg to the vertical and in the plane of the slit. During the burning the operator attempts to keep the hot spot on the side of the carbon next to the slit.

The light from the sample electrode and its hot spot is rendered parallel by a quartz lens of 7 cm focal length and illuminates the slit at a distance of about 60 cm from the source. The width of the slit is about 10 μ. A diaphragm with a square opening about 2.5 cm on a side is used between the prism and focusing lens.

(b) Measurement of Intensity. The spectrum is recorded on 35-mm high-contrast positive motion-picture film. The relative intensities of lines are determined by a rotating-sector method. A half-cylinder sector, 8 mm in diameter, is placed directly before the slit. The intensity of a spectrum line is inversely proportional to the distance between the two extinction points. This distance is read with a 10-power magnifier with scale. When this type of measurement is made with an A.R.L.-Dietert projection comparator-densitometer, which has a 20-power magnification, it is preferable to use a sector 4 mm in diameter. Calibration curves based on conventional transmission measurements may also be worked out with this densitometer.

(c) Spectrum Lines. The spectrum line used for uranium determination is the unresolved group of three lines, 2882.6, 2882.7, and 2882.9A. The internal standard is 5 μg of Pt as $PtCl_4$ solution, and the line 2650A is read. Possible interfering lines are those of Si, 2881.6, but only when very strong; Cu, 2882.9, when 10 μg or more is present; V, 2882.5, which is present in the unpurified carbons but removed by the acid; and Mn, 2882.9, when greater than 25 μg. Ordinarily occasional trouble is experienced with the Si and Cu.

(d) Enhancing Agents. Two compounds are currently used to increase the sensitivity of the spectrographic method and also to serve as spectroscopic buffers. The amounts are not critical; about 10 mg is added to the carbons with a small spoon. The two substances are RbCl and a mixture of equal parts of KCl and $K_2S_2O_7$. The elements Rb and K are the active parts. The latter substance gives greater sensitivity and is easier to procure but seems to produce more background than the RbCl, and the calibration curve with it has a smaller slope in the range of large amounts of uranium.

These agents are used in the following manner: With samples in solution, the RbCl is added to the carbon first, and then the solution is dried in. If $K_2S_2O_7$ is used, about 5 mg of KCl is placed in the electrode first, the solution dried in, and then the mixture of KCl + $K_2S_2O_7$ added. Solution samples from ether extractions are also done this way. With samples in the form of dry ash, either agent is mixed with the sample before placing in the crater. With bone samples and RbCl, however, it is necessary for highest sensitivity to add finally a few drops of HCl and dry.

(e) Standard Samples. Fluid samples such as urine may be given a preservative, and known amounts of uranium are added as a nitrate or acetate solution. Tissue and bone samples are ashed and dissolved in 1 : 1 HNO_3; known amounts of uranium are added in solution, and the mixture is dried and reheated in the furnace.

Materials from unexposed animals are used, and they give a blank for uranium when the direct determination is made. The ether-extraction procedure has not yet been applied to measurement of the amount of uranium normally present in the animals of this locality.

(f) Calibration Curves. Calibration or working curves have been set up from the ratio of the intensity of uranium to the intensity of Pt against the amount of uranium in the standard samples. Different curves are obtained for varying amounts of tissue ash present and for different enhancing agents. The same curves are found to apply fairly well to all types of tissue ash. Ordinarily the amount of extraneous material coming through the ether extraction is so small that the curve for pure solutions is used. These curves may readily be reproduced from the values given in Table 2.3.

Table 2.3 — Ratio of Intensity of Uranium to the Intensity of Platinum and Amount of Uranium in Standard Samples

	Intensity of uranium/intensity of platinum		
Uranium, μg	Pure solution	10 mg ash	30 mg ash
(With RbCl)			
1.0	0.50	0.40	0.16
4.0	1.03	0.90	0.65
8.0	1.53	1.40	1.27
16.0	2.60	2.50	2.45
(With KCl + $K_2S_2O_7$)			
0.1	0.40	0.20	
0.5	0.90	0.55	
1.0	1.15	0.75	
2.0	1.50	0.93	
4.0	1.90	1.15	
8.0	2.30	1.50	

(g) Sensitivity of the Direct Method. The smallest amount of uranium measurable, using all the above-described techniques except the enhancing agents, and in pure solution, is about 10 μg. With RbCl the amount is about 0.5 and with $K_2S_2O_7$ about 0.1 μg. The sensitivity in practice is, respectively, for the soft tissues about 1 and 0.2 μg/g; for bone, 50 and 15 μg/g; and for urine, about 0.3 mg/liter when $K_2S_2O_7$ is used.

(h) Errors in the Direct Method. The standard deviation of a single determination with pure solutions is about ±10 per cent. With ash present or for amounts near the limit of detection the error may be ±17 per cent. The errors, however, are inherently of a random nature and are associated with the burning of the arc and the visual reading of the relative intensities. The error of the result may be correspondingly reduced by making several spectrograms and computing the average.

4.2 Concentration by Ether Extraction. The basic principles of the ether extraction of uranium from a solution saturated with calcium nitrate have been obtained from several project reports. Techniques were then developed for separating uranium from various biological materials, particularly urine, bone, and blood. In order to obtain complete recovery of the uranium in one extraction, however, it is necessary to follow fairly accurately the detailed procedure given below, particularly with regard to the amounts of biological material and the reagents used.

(a) Human Urine. 1. Transfer 25 ml of urine to a 50-ml pyrex beaker and add 2 ml of concentrated HCl and 3 ml of concentrated HNO_3, and a few drops of caprylic alcohol to prevent foaming. If the urine has been standing without preservative or has a precipitate, the collection vessel should be rinsed out with HNO_3, or acid should be added to the urine before sampling.

2. Heat gently on a hot plate to digest the urine and to reduce the volume to about 10 ml.

3. Add 8 g of $Ca(NO_3)_2.4H_2O$ and continue heating until a volume of 12 ml is reached. The calcium nitrate should be kept in a dry state to make accurate weighing possible.

4. Allow to cool slightly and transfer to a 60-ml extraction funnel. Add 3 ml of saturated calcium nitrate solution to the beaker, heat, and transfer the rinse to the funnel. Allow contents to cool in room or under the tap to room temperature.

5. Add 15 ml of diethyl ether, stopper, and shake for 2 min. Discard the lower layer

6. Pour the ether out of the mouth of the separatory funnel into another 60-ml funnel. Take care not to include any water residues left in the bottom of the funnel. Add 15 ml of distilled water to the ether and shake for 2 min.

7. Run out the lower or water layer into a 50-ml beaker, add 1 ml of concentrated HNO_3, evaporate to a volume of 5 to 7 ml, transfer with rinsings to a graduated tube, and make to 10 ml. The solution is then analyzed for uranium by the direct method for pure solutions.

With unknown samples it is convenient to take half this solution for the first test. The smallest concentration readily measurable is thus about 0.04 mg/liter using RbCl and 0.01 mg/liter using $K_2S_2O_7$. The method has been found valid for concentrations between 0.01 and 20 mg/liter. The standard error for a single determination is about ±17 per cent. Faulty technique, however, may produce low recoveries.

(b) Bone. 1. Place a weighed amount of bone ash with acid and water in a 50-ml pyrex beaker and heat gently until all the bone is in solution. The proper proportions of bone and reagents are given in Table 2.4.

2. The remainder of the procedure is the same as that given previously for urine. It is helpful in showing the interface to add 2 drops of 0.1 per cent methyl-red indicator in the fourth step.

The method has been used successfully with recoveries better than 95 per cent on amounts of uranium between 1 and 100 μg in a gram of bone ash, and it may well be much more sensitive than that. There is no difference between using bone standards as described or simply adding uranium to blank bone ash in solution without heating in the furnace.

Table 2.4—Proportions of Bone and Reagent

Wt. of bone ash, g	Vol. of H_2O, ml	Vol. of HCl, ml	Vol. of HNO_3, ml
0.1 – 0.2	7.0	1.0	2.0
0.5	5.5	1.5	3.0
1.0	3.5	2.5	4.0

The errors are about the same as in the urine procedure except that with faulty technique very poor recoveries may be found. The HCl is used in the procedure for digestion purposes and is thus not necessary with the bone but is to be preferred.

(c) Blood. 1. To a 100-ml pyrex beaker containing 10 ml of concentrated HCl and 15 ml of H_2O, add, with stirring, 10 ml of whole blood. Add 10 ml of concentrated HNO_3, several drops of caprylic alcohol, and heat gently with stirring. When the volume is reduced to about 25 ml, transfer rapidly to a 50 ml beaker without rinsing.

2. Continue heating until volume is about 10 ml. Skim off any fat; add 2 ml of H_2O and 5 g of calcium nitrate. The remainder of the procedure is the same as outlined in preceding paragraphs on urine.

The method for blood is tentative with regard to the exact amount of reagents used. Also it has not been tested for maximum sensitivity. Good recoveries, however, have been obtained for amounts of uranium between 10 and 100 μg/10 ml.

4.3 Summary. Methods are given whereby uranium in any biological material may be extracted with ether and determined spectrographically with a sensitivity of 0.1 to 1.0 μg/g and a standard error of less than ±20 per cent.

5. THE SEMIMICRO VOLUMETRIC DETERMINATION OF URANIUM*

For the determination of uranium in relatively pure solutions in amounts ranging from 0.5 to 10 mg, a rapid volumetric method has been devised. The method is based on the precipitation of hexavalent uranium by 8-hydroxyquinoline (Eq. 1). After filtering and thoroughly washing the precipitate to remove excess of precipitant, the uranyl hydroxyquinolate is dissolved in hydrochloric acid (Eq. 2) and treated with a measured excess of standard potassium bromate-bromide solution (Eq. 3). The 8-hydroxyquinoline is quantitatively brominated by the bromate-bromide mixture in the acid medium and forms 5,7-dibromo-8-hydroxyquinoline (Eq. 4). The excess bromine is then determined iodometrically (Eqs. 5 and 6).

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* Method developed by John F. Flagg and Ralph Lobene.

$$UO_2^{++} + 3C_9H_6NOH \rightarrow UO_2(C_9H_6NO)_2.C_9H_6NOH + 2H^+ \qquad (1)$$

$$UO_2(C_9H_6NO)_2.C_9H_6NOH + 2HCl \rightarrow UO_2Cl_2 + 3C_9H_6NOH \qquad (2)$$

$$KBrO_3 + 5KBr + 6HCl \rightarrow 3Br_2 + 3H_2O + 6KCl \qquad (3)$$

$$C_9H_6NOH + 2Br_2 \rightarrow C_9H_4Br_2NOH + 2HBr \qquad (4)$$

$$Br_2 + 2KI \rightarrow 2KBr + I_2 \qquad (5)$$

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI \qquad (6)$$

It is seen that 1 atom of uranium is equivalent to 3 molecules of 8-hydroxyquinoline. But as each molecule of 8-hydroxyquinoline is equivalent to 2 molecules of bromine, 1 atom of uranium is equivalent to 6 molecules, or 12 atoms of bromine. The equivalent weight is thus the atomic weight of uranium divided by 12, or 19.84. Hence 1 ml of 0.1N $KBrO_3$ is equivalent to 1.984 mg of uranium; since the precision of volumetric measurements can readily be made 0.01 ml, the method should determine uranium with a precision of 0.02 mg.

5.1 Reagents. (a) 8-Hydroxyquinoline Solution. Prepared by dissolving 14 g of the solid (obtained from the Eastman Kodak Company) in 100 ml of glacial acetic acid and diluting to 1 liter with distilled water.

(b) Standard Potassium Bromate-Bromide. Prepared by dissolving 2.78 g of Reagent Grade potassium bromate and 25 g of Reagent Grade potassium bromide in 1 liter of water. The solution is standardized by titration against pure arsenious oxide in hydrochloric acid solution, using methyl orange as indicator.

(c) Sodium Thiosulfate, 0.1N. Prepared in conventional manner and standardized against the potassium bromate solution.

5.2 Procedure. The solution to be analyzed should contain from 0.5 to 10 mg of uranyl ion in a total volume of 5 to 20 ml and should be free from all ions except the alkaline earths, alkali metals, and ammonium salts. Two milliliters of 95 per cent ethyl alcohol is added (to prevent precipitation of free 8-hydroxyquinoline), followed by dilute sodium hydroxide until a precipitate forms; then several more drops of base are added. The pH, as shown by pH indicator paper, should be about 5. After warming the solution a few minutes to flocculate the precipitate (which should be a distinct orange color), the uranyl hydroxyquinolate is collected on a filtering crucible of fine porosity. The precipitate is washed with 25 to 50 ml of hot water and dissolved in a few milliliters of warm dilute hydrochloric acid, and the solution is drawn by suction into the clean iodine determination flask in which the final titration will be made. The crucible should be rinsed with 50 to 100 ml of hot water.

To the solution in the iodine flask is added 10 ml of concentrated hydrochloric acid. After cooling flask and contents, the stopper is inserted, and 10.0 ml of 0.1N bromate solution placed in the cup. The stopper is lifted gently to allow most of the bromate to flow into the flask and then replaced to prevent escape of bromine.

Five milliliters of 10 per cent potassium iodide solution is then placed in the cup and after 2 min is allowed to flow into the flask. The cup and stopper are washed well with water before performing the titration. The liberated iodine is titrated with thiosulfate, using a 10-ml microburet, and starch is added near the end point.

Total milliequivalents of bromate used less milliequivalents of thiosulfate gives the milliequivalents of uranium. One milliequivalent of uranium is 19.84 mg.

5.3 Discussion. The average error on a series of samples containing from 2.5 to 10.2 mg of uranium was found in trial experiments to be about 1 per cent. In the range 0.5 to 1.0 mg, the average error is about 3 per cent.

Some critical points in the procedure that will affect the results are here cited:

1. Proper pH for the precipitation. If the pH is too high, there may be some dissolving of the precipitate due to the action of the carbonate ion, formed when carbon dioxide of the air reacts with the alkaline solution. If the pH is much less than 5, precipitation will be incomplete; the optimum range is from 5 to 7.

2. Contamination of the precipitate by excess 8-hydroxyquinoline must be avoided. The presence of alcohol in the solution during the precipitation and adequate washing of the precipitate will minimize the positive errors arising from this source.

3. Loss of bromine in the bromination procedure must be avoided by observing proper precautions in matter of technique.

As this method covers a concentration range normally handled by colorimetric methods, a few points of comparison between the two methods are here cited:

1. Convenience. The volumetric method requires highly accurate standard solutions but a minimum of apparatus. The colorimetric

method is more easily standardized, although it requires more in the way of equipment.

2. Speed. A maximum of 30 min is required for the volumetric determination; a comparable time is required for the colorimetric determination.

3. Accuracy. In general the volumetric method is more accurate than the colorimetric method. The inherent error is about 0.02 mg, which on a 0.5-mg sample is 4 per cent but on larger samples is much less.

PART B. METHODS FOR DETERMINING FLUORINE

The problem of fluorine analysis in connection with toxicological studies was essentially one of determining rather small amounts of fluorine, as fluoride ion, in a variety of substances including air, tissues, biological fluids, and various chemical compounds. The procedures devised were then micromethods, applicable for the analysis of microgram quantities of fluorine. The problem of analyzing high concentrations of fluorine or fluorine compounds was not encountered as was the case in the various plants.

The fluorine methods employed include (1) thorium nitrate titration methods, (2) an enzymatic method, (3) a polarographic method, and (4) a direct bleaching method using ferric salicylate.

6. THORIUM NITRATE TITRATION*

Two variations of this method were in use, depending upon the type of sample to be analyzed. The "long method" was used for samples of uncertain origin or those containing much organic material. The "short method" was used in those cases where the fluoride was relatively free from contamination.

6.1 Long Method. This method depends on the following reactions: Thorium nitrate, $Th(NO_3)_4$, combines with alizarin, an organic dye, in water solution to form a pink-colored compound. The color of this thorium-alizarin compound, or lake, is destroyed by fluoride ion, which combines with the thorium to form a complex thorium fluoride ion, ThF_6^{--}. Thus the bleaching of the color may be used for the quantitative determination of fluoride, and, as the reaction is very sensitive, it may be used on the microscale.

Many different types of fluoride-containing materials may be analyzed by this method. Of particular interest have been biological materials such as urine, hard and soft tissue, blood, and animal feeds.

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* Method developed by Ann Tarbell, Marion J. Voss, and Frank A. Smith.

The method is not limited to such substances, however, and may be used successfully for the determination of fluorine in a great variety of substances containing small quantities of the element.

In all cases save those in which pure fluoride solutions are to be analyzed, a preliminary separation of the fluorine must be made. The first step in the preliminary treatment consists of an ashing, in the presence of lime, which destroys organic matter. The lime serves as a fixative for the fluoride ion, combining with it to form calcium fluoride. The ash is then transferred to a perchloric acid solution that contains a little silver perchlorate to precipitate any chloride ion as silver chloride, and the solution is distilled to remove the fluoride, as described by Willard and Winter.* If chloride were not removed, some chlorine might distill over with the hydrogen fluoride and cause erratic results in subsequent steps. The distillate, then, from this step contains fluorine as fluoride ion in relatively pure form. Occasionally the distillate may be slightly colored; this is often encountered in the analysis of blood samples. In such cases the solution is evaporated in the presence of lime and redistilled.

An aliquot of the distillate is neutralized and treated with a little hydroxylamine hydrochloride to destroy chlorine; it is then ready for titration. The pH is adjusted to 2.75 with hydrochloric acid, and the alizarin indicator is added. Thorium nitrate is added from a buret until a pink color is produced, i.e., until an excess is present. The solution is then transferred to a Nessler tube. To a blank containing double-distilled water in place of the fluoride distillate but all other reagents as in the fluoride solution, the same amount of thorium nitrate is added. This solution is then titrated with standard sodium fluoride until the colors of sample and blank, when compared under identical conditions, match. From the volume of standard solution added, the amount of fluoride in the sample may be calculated. Appropriate corrections are made for any blank on the reagents.

The recovery of fluoride in amounts less than 100 μg is from 95 to 100 per cent in the case of pure solutions, and from 90 to 95 per cent in the case of urine samples to which known amounts of fluoride had been added. For the actual analysis, 3 to 30 μg is a convenient amount of fluoride titer.

Great care must be exercised to prevent contamination, as the method is so sensitive that even minor traces of fluorides will cause erratic results. All operations should be carried out in atmospheres free from fluorine-containing substances. Rubber connections, etc., in apparatus should be eliminated wherever possible, as the new synthetic rubbers contain appreciable amounts of fluoride. Natural rubber may be used safely, however.

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*H. H. Willard and O. H. Winter, Ind. Eng. Chem., Anal. Ed., 8: 384 (1933).

The method is moderately simple to perform, and no special equipment is required beyond stills, platinum crucibles for ashing, well-illuminated stands for making color comparisons, and the usual microvolumetric equipment. When multiple stills are used, two workers, given 12 stills and working 8 hr per day, can perform about eighty analyses per week.

The method is slow in the event a large number of samples varying widely in range of fluoride content are to be analyzed. This results from the necessity of taking several aliquots of the fluoride distillate to find the proper concentration for making the final titration. The short method, described in Sec. 6.2, may in certain cases be of value in securing greater speed.

(a) Reagents. Calcium oxide, c.p., "Special fluorine free," from Fisher Scientific Company, contains from zero to 0.00065 per cent fluorine (as F). This is preferred to magnesium acetate, as the lime is easier to handle and has been found lower in fluorides than the magnesium acetate obtained.

Anhydrous silver perchlorate, Reagent Grade, from G. Frederick Smith Chemical Company.

Perchloric acid, 70 to 72 per cent, Baker's C. P. Analyzed. No purification has been found necessary for this reagent as received.

Hydrochloric acid solution, 0.05N.

Potassium hydroxide solution, 1.0N; also 0.05N.

p-Nitrophenol indicator, 0.25 g in 25 per cent ethyl alcohol.

Hydroxylamine hydrochloride, 1 per cent aqueous solution.

Sodium alizarin sulfonate indicator, 0.015 g in 100 ml of water made up once a week.

Thorium nitrate solution, 0.1840 g $Th(NO_3)_4.4H_2O$/liter.

Sodium fluoride solution, a special sample of spectroscopically pure salt, 20.2 mg/liter; 1 ml of this solution is thus equivalent to 10 μg of fluoride ion. A specimen of Baker's salt was unsatisfactory. If no pure standard is obtainable, it is recommended that a sample of potassium silicofluoride be prepared.

(b) Special Apparatus. 1. Overhead radiant heater for evaporating and charring. This is an extremely useful piece of equipment, as urine samples can be safely evaporated and charred in 60 min with a minimum of attention.

2. Muffle furnace, Hosking type FD 204, pyrometer, and rheostat. A perforated stainless-steel tray on legs 2 ¼ in. high with stainless-steel weld may be used to double the capacity of the furnace, allowing room for at least 24 dishes.

3. Platinum dishes, 65 to 75-ml capacity. These should be cleaned after each determination by boiling in dilute HCl and igniting over a Méker burner. Heavily glazed porcelain dishes may be used for water samples; silica dishes are satisfactory for analysis of many types of aqueous solutions. Blanks should be done on these. Small nickel combustion crucibles, Kawin capsule form, are convenient for ashing small amounts of dry material such as bones and teeth. If platinum dishes are not available, it is possible that gold dishes might be found suitable. Silver is said to be unsatisfactory, and nickel, although it can be used for dry samples, oxidizes and flakes badly when heated during the ashing of a large organic sample. All dishes should be cleaned with dilute HCl followed by ignition or heating at 600°C.

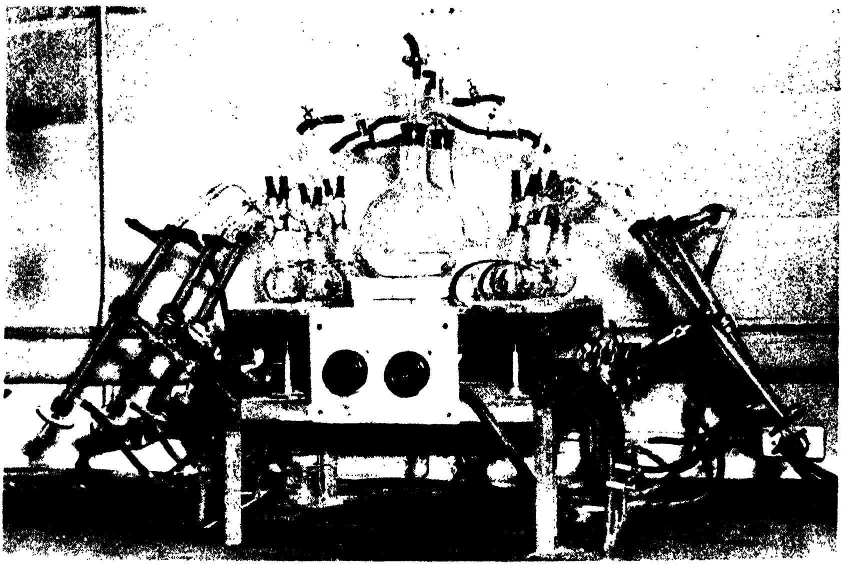

Fig. 2.13—Sextuple stills and rack.

4. Sextuple stills and rack of special design (see Fig. 2.13). These stills embody ideas derived from the Kettering Laboratory, the laboratories of the Aluminum Company of America, and the report by Clifford[1] and have been modified for greater speed and convenience. The stills should be of pyrex glass, and blank determinations should be run until a constant and negligible blank is obtained for each still

(less than 1 mg). A new still may yield high values at first, but several boilings with perchloric acid will in most cases reduce the blank. If a still is ever found that "leaks" fluoride continually, it should be discarded. The "necks" of the still head should fit the steam inlet tube and thermometer to give as close a glass-to-glass fit as possible. This design of the still head has proved satisfactory in that recoveries of fluoride are good, and the distillation of phosphate and excessive perchloric acid, both of which interfere in the titration, is reduced to a negligible minimum. The passage of the steam inlet tube and thermometer through the T-s 24/40 joint creates an effective baffle that prevents AgCl and liquid from splashing over. All rubber tubing connections should be made of well-boiled, prewar natural rubber tubing. The synthetic tubing now obtainable has given large and erratic distillation blanks.

5. Nessler tubes, tall form graduated at 50 to 100 ml with ground-glass caps. These may be obtained from Scientific Glass Company.

6. Comparator block for Nessler tubes, illuminated by a daylight fluorescent lamp.

7. A set of porcelain spoons of varying sizes. These can be calibrated to measure a fixed weight of dry reagent such as lime or $AgClO_4$ and save time otherwise required by weighing.

8. In addition there are required the usual volumetric glassware, microburets of 5-ml capacity, and volumetric receiving flasks made from Florence flasks calibrated to contain 125 ml.

(c) Procedure. Urine analysis will be described in detail; other samples may be distilled and titrated in the same way. Modification in preparation or ashing may be required according to the nature of the specimen.

The urines analyzed may be spot samples of portions of a 24-hr output. It has been demonstrated that, over a period of time, analyses of spot samples agree satisfactorily with 24-hr values.

Fifty milliliters of urine is poured into a platinum dish containing 0.5 to 1.0 g of CaO (measured by a spoon), evaporated to dryness on a hot plate or under an overhead heater, and charred to a black ash. It is then ashed in a muffle furnace at 570°C for 4 to 6 hr or until the ash is almost white. A small amount of unburned carbon does not interfere.

The cooled ash is ground to powder by means of flattened glass stirring rods and transferred through a powder funnel into the 100-ml distilling flask, which contains a piece of carborundum, several glass beads, and approximately 2.6 g of $AgClO_4$, which is slightly in excess of the amount required to precipitate chloride in 50 ml of normal urine. The ash that sticks to the platinum dish is washed into the flask with the aid of the smallest possible amount of water and a few drops of $HClO_4$. The glass joints are wet with water and sealed. When all the stills are loaded, 10 ml of $HClO_4$ is added to each through the flared end of the steam inlet, the latter is rinsed down with water, the steam tube connected (clamp closed), and the excess water distilled off until the temperature reaches 135°C. At this point, steam is admitted from the boiling generator. The flame below the flask and the steam flow are adjusted to maintain a temperature of 135 to 137°C. All six stills can be easily kept in this range. One operator can handle two sextuple racks at one time. Distillation is continued at an even rate until 125 ml is collected; this requires approximately 1 ¼ hr. Clumping of the AgCl precipitate may occur at first, but this is broken up by the action of the steam. The spring brass clamps permit shaking of individual stills without straining the glass joints. After the run, the distillates are stoppered with clean, boiled rubber stoppers and preferably titrated within a few hours. The glass stills are cleaned immediately by thoroughly rinsing with hot tap water and distilled water. If they are kept in almost constant use, no further treatment is necessary. Where an amount greater than 1 mg has been accidentally distilled, the still is boiled out with 20 per cent NaOH followed by fuming out with $HClO_4$ to preclude any contamination of the next analysis.

The titration is the usual back-titration method involving the use of an individual blank for each sample and is based on the fading of the red-purple thorium alizarin sulfonate lake by fluoride ion. The titration can be conveniently done in lots of six samples. Twelve 125-ml Erlenmeyer flasks are set out, one each for sample and blank. An aliquot of distillate containing approximately 15 to 30 μg of fluoride is placed in the sample flask, and water is added to 40 ml. The blank flask contains 40 ml of water, and to each flask is added 0.05 ml of 1.0N KOH plus 2 drops of *p*-nitrophenol indicator solution. In most cases where the distillation has been carefully carried out this is enough KOH to nautralize a 40-ml aliquot. If excess acid is present, it can be neutralized by addition of more KOH to both sample and blank. If more than 0.2 ml of KOH is required, it is advisable to evaporate the distillate to a small volume in the presence of CaO and redistill, as large amounts of $KClO_4$ interfere.

Each flask is then carefully neutralized to the indicator (faint tinge of yellow) with 0.05N HCl, and to each is added, with swirling, 1.0 ml of hydroxylamine hydrochloride solution to reduce any traces of free Cl_2. This is followed by 1.0 ml of alizarin indicator and 2.0 ml of 0.05N HCl. This should give a pH of 2.75. It is very convenient to add these reagents from self-filling burets mounted in order upon a combination bottle and buret stand fixed to the titration table. Thorium nitrate solution is then run into the sample flask from a microburet until a pale-pink color is formed, and the solution is poured into a Nessler tube. The flask is rinsed with 5 ml of water; the rinsings are added to the tube and mixed in by gentle inversion. Exactly the same amount of thorium nitrate is added to the blank flask, and the standard fluoride is then run in from a microburet until the pink color matches that of the sample.

The final check is made by comparing the colors in the Nessler tubes observed against a white light. The amount of fluoride (which can be read off directly from the buret) added to the blank is the amount of fluoride contained in the sample aliquot. A simple calculation and correction for the reagent blank (chiefly CaO) will then give the milligrams of fluoride per liter of urine.

It has been the custom occasionally to analyze a sample of tap water obtained at the same time of the urine collection. This offers a convenient check for contamination during collection as well as during analysis and illustrates the range of urinary fluoride to be expected from intake of public water supplies.

Hard tissue such as teeth and bones may be analyzed in much the same manner. Thoroughly dried specimens may be analyzed whole or powdered in a diamond mortar, weighed into nickel crucibles, and ashed for 12 to 18 hr. Hard tissue prepared by the glycol ashing procedure of Crowell *et al.*[2] may be distilled directly.

Large volumes of aqueous solutions may be evaporated to small volume in presence of lime in silica or platinum dishes and washed into the stills. For evaporation the Fisher Infra Radiator has been satisfactory.

6.2 Short Method. In the routine determination of fluoride ion in large numbers of urine samples of varying concentration, the back-titration procedure (Sec. 6.1), although it gives satisfactory results, is quite time-consuming. The so-called "short method" was developed to meet this objection.

This method also depends on the formation of the thorium fluoride complex but in a manner slightly different from that of the long method. The solution containing fluoride (usually a distillate, obtained in the manner described previously) is adjusted to pH 3 with chloroacetic acid buffer, and chrome azurol S is added. The dye imparts a pink color to the solution, the color persisting during the titration with thorium nitrate until the end point is reached. Then the pink changes to the blue color of the thorium lake of the dye. The color is matched in a Nessler cylinder with a blank or with a permanent lavender standard prepared from copper and cobalt salts.

Using this method, a range of 1 to 100 μg of fluoride is easily covered without taking more than one sample. The average error, on a series of samples of from 3 to 30 μg, was about 0.2 μg. Some interfering substances that may lead to errors are potassium chloride, sulfate, and acetate, as well as uranyl salts. Potassium nitrate, borate, and carbonate do not interfere.

The method has been used in the analysis of biological samples; also in the analysis of air samples (for hydrogen fluoride). The increased speed is apparent when one notes that by the long method about four solutions per hour can be titrated, whereas by this method the number is about fifteen, with no decrease in accuracy.

(a) Reagents. All reagent solutions and all dilutions were made routinely with double-distilled water. The development and precipitation of the thorium lake appeared to be so sensitive to foreign substances that this precaution has been maintained.

Chrome azurol S may be secured from chemical manufacturers.

The buffer employed was that commonly used in titrations of fluoride ion with thorium nitrate. The use here of KOH instead of NaOH was dictated by the belief that in the presence of potassium salts the end points were more easily read in both this and the back titration. The buffer when used in this procedure gave a final pH of 3.1 ± 0.05 units.

Distilled water, redistilled from alkaline permanganate.

Potassium hydroxide, 1.0N.

p-Nitrophenol indicator, 0.5 per cent solution in 25 per cent alcohol.

Perchloric acid, 0.05N.

Chrome azurol S, 0.02 per cent aqueous solution.

Potassium hydroxide-chloroacetic acid buffer. Dissolve 22.7 g of $CH_2ClCOOH$ in water and dilute to 100 ml. Titrate 50 ml with 6N KOH to neutralize, combine the two portions, and dilute to 1 liter.

Thorium nitrate solution, 0.200 g $Th(NO_3)_4.4H_2O$ per liter of solution.

Copper nitrate-cobalt nitrate as permanent lavender standard.

(b) Procedure. Pipet an aliquot containing 0.3 to 40 μg of fluoride ion into a glass vial 80 by 25 mm and dilute to 10 ml with distilled water.

Add 0.020 ml of 1.0N KOH. If the solution is already in KOH, adjust the aliquot to give the equivalent of 0.02 ml of 1.0N KOH. Add 1 drop of *p*-nitrophenol indicator, and neutralize with 0.05N $HClO_4$. Add 0.25 ml of dye solution from a buret. Add 0.25 ml of buffer solution.

Titrate with thorium nitrate solution from a 5-ml buret graduated in 0.01 ml, and match against the permanent lavender standard.

Read the amount of fluoride ion present from a standard curve.

(c) Discussion. Choice of Aliquot. A 10-ml volume is conveniently manipulated in a vial of the size used. The aliquot has to be chosen both for concentration of fluoride ion and of potassium ion. It is important to maintain accurately the concentration of the latter, as the salt concentration has a marked influence on the titration (see below). As 0.20 ml of 1.0N potassium hydroxide was sufficient to neutralize 10 ml of distillate obtained by the techniques described previously (Sec. 6.1c), this amount was chosen.

Adjustment of pH. Use *p*-nitrophenol indicator, which fades to colorless at the approximate point of change of chrome azurol S from yellow to pink. The dye itself may, in fact, be used as the sole indicator of pH; thus the addition of *p*-nitrophenol may be omitted, and the potassium hydroxide may be neutralized to the turning point of chrome azurol S.

Permanent Standard. The sharpest color change from the pinkish-orange color of the solution to the bluish purple of the lake occurs with the addition of 0.1 to 0.12 ml of thorium nitrate reagent. The blank is then prepared by the addition of 0.1 ml of reagent, and upon overtitration by 0.01 to 0.02 ml of reagent, the end point is very strikingly defined. On standing for 30 to 60 sec, the solution turns a deeper blue; after 24 hr, the lake precipitates.

To eliminate the time factor a permanent standard is prepared. Copper and cobaltous nitrates are mixed so as to match a fresh blank, and the permanent standard thus obtained used for further comparison purposes. Titrations should be made on white backgrounds, by a daylight lamp.

Standardization of the Titration and Accuracy of Results. The thorium nitrate solution is standardized against a solution of pure sodium fluoride containing 10 μg of fluoride ion per milliliter, and from this a calibration curve is prepared. In general, any trained operator can read the end point satisfactorily. Results taken at random from titrations of pure solutions by one operator show a maximum error of -0.6 μg (-3.1 per cent), and the average error is ± 0.2 μg.

Interfering Compounds. A number of common substances added to the solution to be titrated produce the following effects: 1 ml of ethyl alcohol and the substitution for $KClO_4$ of equivalent quantities of KNO_3, $K_2B_4O_7$, and K_2CO_3 have no effect. Potassium chloride, sulfate, and acetate interfere in amounts of a few milligrams each. Uranyl ion forms a lake with the dye.

Performance Time. The short method is much more rapid than the previous back-titration method. By the latter procedure an experienced worker may be able to titrate four solutions of average concentration (5 to 20 μg) in an hour, whereas by the short method an operator can complete about fifteen determinations in the same length of time.

Determination of Hydrogen Fluoride in Air. Samples of air-HF mixtures have been collected in 0.005N potassium hydroxide, and the fluoride concentration has been determined by both the direct titration and distillation–back-titration techniques. Agreement between values found for 18 samples is good, well within the limits (±10 per cent) for determinations by the latter procedure in the range covered (1 to 2 μg/ml).

7. THE ENZYMATIC DETERMINATION OF FLUORIDE*

The specific inhibition of enzymatic activity by fluoride ion forms the basis of this method for the quantitative determination of fluoride. A direct relationship exists between the concentration of fluoride ion and inhibition in enzymatic activity caused by it. Thus from an empirical standard curve of relative inhibition (or activity) vs. fluoride concentration the concentration of fluoride in "unknown" solutions can be ascertained.

The method has been adapted for the determination of fluoride ion in pure aqueous solution and in aqueous solution where uranium salts are contaminants.

7.1 Materials. (a) Enzyme. Hog-liver esterase serves as the enzyme for the method. Both crude preparations as well as more highly purified ones have been used. The enzyme solutions are stabilized with glycerol (50 to 80 per cent by volume).

(b) Substrate. Ethyl butyrate (b.p. 119 to 121°C, Eastman).

(c) Standard Fluoride Solutions. Prepared from sodium fluoride (Baker's, C.P., Analyzed).

(d) Standard Alkali. Sodium hydroxide, 0.050N.

7.2 Procedure. Aliquots of standard solutions of fluoride and ethyl butyrate (whose concentrations are, however, unknown to the analyst working with them) are introduced into an Erlenmeyer flask containing distilled water. The mixture is brought to the phenolphthalein end point (3 drops of 0.2 per cent phenolphthalein in 40 per cent ethyl alcohol) by the addition of sodium hydroxide. An additional volume of sodium hydroxide equivalent to the titratable acidity of the enzyme solution (previously determined) is added and finally 1 ml of the enzyme solution is introduced over a 10- to 13-sec period. The final volume of the mixture should be 30 ml. The solution is incubated for 30 min at 25 ±0.5°C. At the termination of this incubation period, 10 ml of cold ethyl alcohol is added to retard the action of the enzyme, and the mixture is titrated back to the phenolphthalein end point. Suitable blanks of enzyme alone and substrate alone are run, and the necessary titration corrections are made.

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* Method developed by Max Schlamowitz.

7.3 Discussion and Results. The volume of standard sodium hydroxide solution required to titrate the butyric acid liberated in the mixture by enzymatic action is a measure of enzymatic activity. The inhibition of enzymatic activity by fluoride is evidenced by a reduction in the amount of butyric acid formed and in the volume of sodium hydroxide solution used to titrate it. A graphic representation of the relationship between volume of 0.0500N sodium hydroxide and the concentration of fluoride ion will yield a smooth curve that asymptotically becomes parallel to the concentration axis. The useful range for the determination of fluoride concentrations is 0.0 to 0.5 ppm by weight. The useful range may be extended to 10 ppm by the addition of 174 ppm of zirconium ($ZrOCl_2.8H_2O$). It is believed that other substances capable of forming slightly dissociated complexes with fluoride would serve as well as this.

The method is accurate to within ±5 per cent of theoretical for buffer-free solutions of fluoride. Analysis of fluoride solutions in the presence of known amounts of uranyl nitrate revealed that concentrations of uranium up to fifty times that of fluoride could be tolerated. Inhibition of enzymatic activity of UO_2F_2 was shown to correspond almost exactly with that calculated on the basis of its fluorine content.

The applicability of the method for the determination of fluoride has been demonstrated for chamber atmospheres containing fluorine. The fluorine in the air samples was absorbed in either water or sodium hydroxide. All analyses were checked against values obtained by the thorium titration method on aliquots of the same sample. The presence of sodium chloride or uranium was without interference, but, in those instances where large amounts of carbon dioxide were absorbed during the air sampling, low results were obtained.

8. THE POLAROGRAPHIC DETERMINATION OF FLUORIDE*

It was observed that the U_4 reduction step at the dropping-mercury electrode was removed by the addition of fluoride ion. This is probably due to the formation of a complex of U_4, which is reduced at a considerably higher potential. It has been observed that nitrate produces a catalytic wave at the potential at which the tetravalent state of uranium is reduced. The wave height was shown to be a function of the nitrate concentration when the uranium concentration is maintained at a specific level. Curve 1a, Fig. 2.14 shows a catalytic wave obtained at a uranium concentration of $10^{-8}M$ and a nitrate concentration of 0.05M. This method has been used to determine uranium in very small concentrations. It is also known that fluoride removes this catalytic wave. Since the nitrate catalytic wave occurs at the U_4 reduction potential, fluoride undoubtedly removes the catalytic wave by complexing U_4. Thus the basis for an amperometric titration was at hand. The unknown fluoride might be titrated with standard uranyl acetate in the polarographic cell. When the amount of uranium that complexes with the fluoride exceeds the concentration of fluoride in solution, the increase in galvanometer deflection changes from a linear to an exponential increase. The point of change is taken as the end point.

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* Method developed by G. H. Tishkoff.

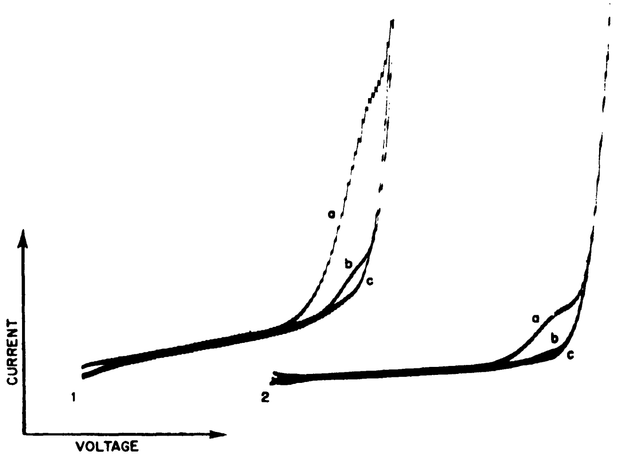

Fig. 2.14—Catalytic wave of 10^{-8} M uranium in 0.05M nitrate. Curves 1 and 2 are identical except that curve 2 was obtained at lower sensitivity of the apparatus. Curve a is 10^{-8} M uranium in 0.05M nitrate. Curve b is 10^{-8} M uranium in 0.05M nitrate in the presence of trace quantities of fluoride. Curve c is 10^{-8} M uranium in 0.05M nitrate in the presence of excess fluoride.

8.1 Preliminary Studies. The effect of nitrate on the U_4 wave in acetate buffer at pH 4.0 was studied, and results are shown in Figs. 2.15 and 2.16. Figure 2.15 shows the normal uranium spectrum in weakly acid solution; it consists of three distinct waves, the third wave corresponding to the reduction of U_4 at a potential of −1.2 volts referred to the saturated calomel electrode. When nitrate is added, the U_4 wave is catalyzed to such an extent that the wave does not appear on the polarogram. This is shown in Fig. 2.16. If the U_4 is responsible for the catalytic behavior in nitrate solution, it would be expected that removal of U_4 in the form of a complex would remove the catalytic wave; indeed, this is the case.

A quantitative study of the effect of fluoride on the U_4 wave was made by adding various amounts of fluoride to a 10^{-4}M uranium solution in 0.1M HCl. In HCl the second and third waves merge; only two waves are observed. Consequently the composite wave is twice the height of the first and consists in part of the reduction of U_4.

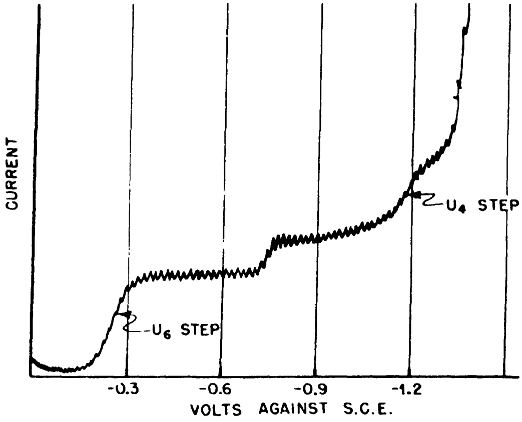

Fig. 2.15—Polarographic waves of uranium in weakly acid solution. 0.001M uranium; 0.1M acetate buffer; pH 4.0.

Curve 1 in Fig. 2.17 illustrates the composite wave. It is seen from curves 2 and 3 in Fig. 2.17 that, after 0.0002M (or greater) fluoride is added, no reduction in the height of the composite wave occurs. This is because the U_4 wave is removed as the fluoride complex, leaving only two waves of equal height. The first wave is of the reduction of U_6 to U_5; the second is of the reduction of U_5 to some lower form. Two equivalents of fluoride are needed per atom of uranium; the compound formed may be UOF_2.

Figure 2.18 shows various effects. Curve 1 is the normal polarographic spectrum of uranium in 0.1M hydrochloric acid. Curve 2 shows the nitrate effect in the absence of fluoride, and curve 3 shows the effect of fluoride on the catalytic wave and is similar to Fig. 2.17, curve 3, except for the distortion of the diffusion current in the second wave. Therefore, if the diffusion current is measured at −0.91 volt against the saturated calomel electrode in a solution of fluoride containing a large excess of nitrate, a linear increase in the diffusion current will result as the uranium concentration is increased. Eventually a point is reached where the fluoride cannot complex with the

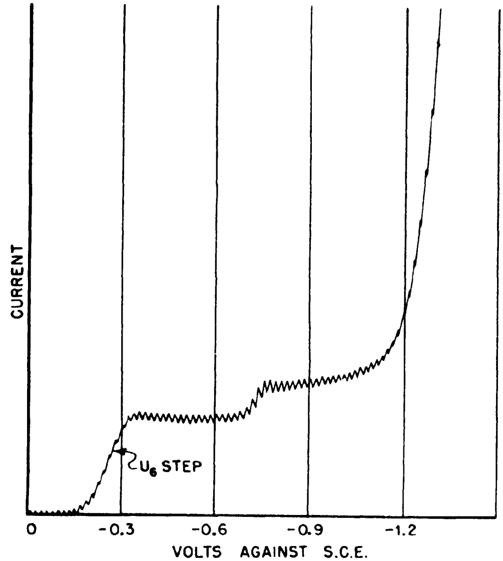

Fig. 2.16—Effect of nitrate on the U$_4$ wave. NO$_3^-$ present; 0.001M uranium; 0.1M acetate buffer; pH 4.0.

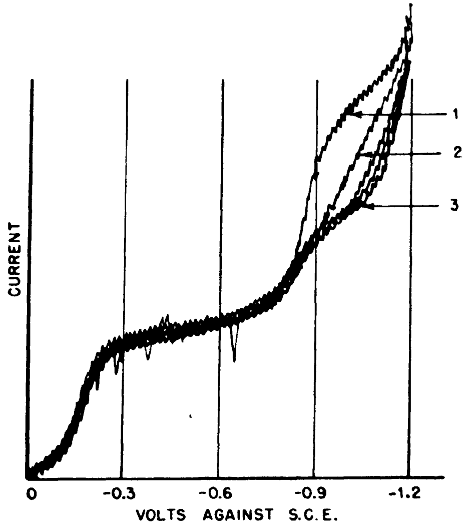

Fig. 2.17—Effect of fluoride on U$_4$ wave. 0.0001M uranium; 0.1M HCl. (1) No NaF added; (2) 0.0001M NaF added; (3) 0.0002 to 0.0005M NaF added.

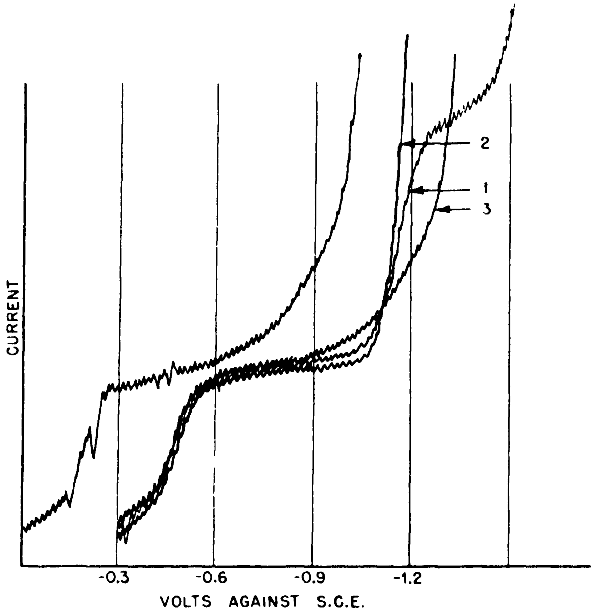

Fig. 2.18—Polarographic spectrum of 0.0001M uranium in 0.1N HCl. (1) No salts added; (2) 0.001M KNO_3 added; (3) 0.001M KNO_3 + 0.0005M NaF added.

entire quantity of U_4 formed on reduction. This change occurs at a fluoride concentration equal to twice the uranium concentration. When the uranium concentration exceeds this value, the diffusion current will increase exponentially. As uranium is added, the second wave changes from a slight wave to a composite wave, which is twice the height of the first wave; the diffusion current also increases. Titration at a constant applied voltage is known as "amperometric titration" and forms the basis for the method.

8.2 Apparatus. The apparatus used is essentially the same as that described in the polarographic literature for titrating in the absence of air. A microburet is attached; it has a capacity of 5 ml and can be read to 0.005 ml.

A Sargent-Heyrovsky polarograph is used to obtain the current-voltage relations.

8.3 Procedure. The buret is filled with a stock solution of 0.002M uranyl acetate in 0.1M hydrochloric acid. The unknown fluoride solution is diluted with sufficient stock solution of HCl and KNO_3 until a solution that is 0.1M in HCl and 0.001M in KNO_3 is obtained. Then 20.0 ml of the solution is pipeted into the polarographic cell, and nitrogen is passed through at a rate of 37 cu cm/min for 15 min. The stock solution of uranyl acetate is then added in increments of 0.2 ml; afterward nitrogen is passed through the solution for 1 to 2 min. The nitrogen is then turned off, the mercury capillary opened, and the galvanometer reading recorded. This procedure is repeated for each addition of uranium. If air is removed from the buret by nitrogen, the procedure is shortened in so far as nitrogen need not be passed through the solution after each addition of uranium.

If no change in slope is observed (plotting current vs. milliliters of uranium solution added) after adding 5 ml, the fluoride solution must be diluted to a concentration less than 0.0004M. After sufficient practice the range can be readily found.

8.4 Calculations. Since the solution is being diluted during the titration, a correction factor must be applied to the galvanometer readings. If V is the volume of fluoride solution in the cell and v is the volume of uranium solution added, then $V + v/V$ is the correction factor.

The galvanometer readings are multiplied by the factor, and the corrected readings are plotted as ordinates against volume of uranium solution added. Sufficient points are obtained to give a straight base line. These are usually the first four or five points. An intersecting line is then drawn through the first two or three points that deviate from the base line. Since the points of deviation increase exponentially, no more than three points should be used in constructing the intersecting line. In this manner, the value of x on the abscissa of the point of intersection is found.

Let x represent the milliliters of uranium solution (found at the point of intersection) that have been added to 20 ml of fluoride unknown. Let y represent the molarity of the fluoride solution. Then

$$\frac{x(0.002)}{20} = z$$

$$2z = y = \text{molarity of fluoride}$$

The value of y must be corrected for dilution that occurred when hydrochloric acid and potassium nitrate were added.

8.5 Results. The sensitivity of the method is 1 μg of fluoride per milliliter; the average error is ±7 per cent. The accuracy could be improved by careful control of temperature.

Interference will arise from the presence of other ions that complex with uranium, such as citrate, acetate, and oxalate, and from any ions that precipitate uranium, such as phosphate.

Further modifications of the method may be suggested, such as titrating with uranyl nitrate in nitric acid medium in order to accentuate the catalytic effect.

In its present stage of development the method is best adapted for the rapid analysis of relatively pure solutions of fluoride ion.

9. FERRISAL METHOD FOR DETERMINING FLUORIDE*

It is known that the ferric-salicylic acid complex is decolorized by the fluoride ion. This complex, which has been called "ferrisal," may be used to cover a range of fluoride concentrations from 1 to 140 μg/ml of solution. It is possible to increase the sensitivity somewhat by diluting the solution of the complex to which the fluoride is added.

In connection with the analysis of hydrogen fluoride in air, at concentrations of from 1 to 10 ppm (or higher), this method was useful. The air sample is drawn through the purple-colored solution of the complex. After a known volume has been sampled, the amount of bleaching in the solution caused by hydrogen fluoride is determined, in most cases by direct reading in a photoelectric colorimeter. Direct comparison with a calibration curve obtained from the bleaching of known amounts of fluoride enables the concentration of hydrogen fluoride in the air to be calculated.

9.1 Reagents. 1. Ferric ammonium sulfate solution, prepared by dissolving 3.220 g of Reagent Grade ferric ammonium sulfate in a mixture of 200 ml of water and 50 ml of 0.1N hydrochloric acid, and diluting to 1 liter.

2. Salicylic acid solution, prepared by dissolving 1.366 g of salicylic acid in 600 ml of water at 80 to 90°C, and when cool diluting to 1 liter.

3. Hydrochloric acid, pH 3.1, prepared by adding 0.1N hydrochloric acid to water in the ratio of 1 ml of acid to 100 ml of water. The pH is checked with a glass-electrode pH meter.

4. Ferric-salicylic acid stock solution (ferrisal), prepared by adding 100 ml of salicylic acid and 80 ml of ferric ammonium sulfate to a 1-liter volumetric flask, then adding 800 ml of water, adjusting to pH 3.1 with sodium hydroxide (0.1N), and making to volume. The solution is stored in amber bottles.

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*Method adapted by John F. Flagg and Ralph Lobene.

5. Ferric-salicylic acid standard solution, prepared by diluting 3 volumes of the stock solution with 1 volume of hydrochloric acid, pH 3.1. A more dilute standard ferrisal solution may be prepared by mixing 1 volume of stock solution with 2 volumes of hydrochloric acid, pH 3.1.

9.2 Sampling Devices. (a) Circulating Bubbler. This device, designed for the sampling of air or other gases containing hydrogen fluoride, is shown in Fig. 2.19. It is arranged to provide circulation of the ferrisal reagent, permit frothing space (frothing reduced by the addition of a small quantity of butyl alcohol), and break up the gas stream by means of the fritted disk. The volume of liquid used in the bubbler should be as small as possible in order to gain the maximum bleaching effect. For a bubbler of the size shown, 90 ml is a convenient volume to use.

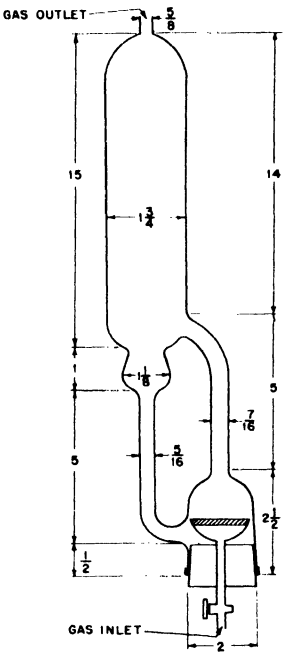

Fig. 2.19—Apparatus for HF absorption in circulating bubbler.

(b) Gas Bulb. This consists of a 5-liter flask, paraffined inside, and fitted with a two-hole paraffined rubber stopper. A separatory funnel (50 ml capacity) is fitted in one hole; a stopcock is fitted in the other for evacuating the bulb. The open end of the separatory funnel is fitted with a one-hole stopper carrying a bent glass tube drawn down to a fine point. The gas sample is allowed to bubble through this tube into the evacuated flask, the absorbing solution being contained in the separatory funnel. When the tube is filled, the solution in the funnel is allowed to run into the bulb, and the whole is shaken for 10 min to ensure complete absorption of the fluoride gas.

With this system, as little as 10 ml of ferrisal can be used. With the circulating bubbler, a larger gas sample can be taken, so that one advantage offsets the other.

9.3 Color Measurement. The Klett-Summerson colorimeter is a convenient instrument for reading the colors of the bleached and unbleached ferrisal solutions. The green filter, 5400A, should be used. The reproducibility of the readings is about 2 divisions.

9.4 Calibrations. Calibration curves are prepared by adding known amounts of sodium fluoride (or hydrogen fluoride) solution to given volumes of the standard ferrisal mixture. The concentration range covered should be from 0 to about 1,000 μg of fluoride per 90 ml of standard ferrisal (assuming use of a circulating bubbler device). It is found that 1 colorimeter division (Klett instrument) will correspond to about 7 μg of hydrogen fluoride.

9.5 Blanks. In analyzing air samples it is well to determine a blank, using air known to be free of fluoride gases. This may not be absolutely necessary, however, as numerous tests have shown that ordinary air, sampled at a rate of 1.2 liters/min for 30 min, produces no detectable bleaching.

9.6 Procedure. The procedure consists simply in passing the gas, at a known rate and for a known time, through a definite volume of ferrisal placed in the circulating bubbler. A volume of 90 ml is convenient; to this is added 1 ml of butyl alcohol to assist in reducing frothing. At the end of the run, the ferrisal is removed from the bubbler and transferred to the colorimeter tubes, and the color is measured. Comparison with the standard chart enables the parts per million of fluoride to be calculated.

If the bulb sampler is used, 20.0 ml or some other convenient volume of standard ferrisal is placed in the separatory funnel. The bulb is evacuated, and then the fluoride gas is allowed to flow slowly in through the separatory funnel until the bulb is at atmospheric pressure. The ferrisal is then drained into the bulb, the system shaken for 10 min, the ferrisal removed, and its color determined.

9.7 Discussion. The sampling rate is important. The higher the concentration of hydrogen fluoride, the lower should be the sampling rate. A rate of 0.5 liter of hydrogen fluoride per minute at a concentration of 10 ppm is considered satisfactory.

The method is capable of accuracy no greater than 10 per cent, at least with the Klett-type colorimeter. Results agreeing within 2 ppm of those obtained by the long method (Sec. 6.1) on chamber samples have been obtained, at about 10 ppm total concentration. The method is best adapted for use in plants or other sites where quick, semiquantitative determination of hydrogen fluoride concentrations is required.

BIBLIOGRAPHY

1. P. A. Clifford, J. Assoc. Offic. Agr. Chemists, 27: 90, 246 (1944).
2. C. D. Crowell, H. C. Hodge, and W. R. Line, J. Dental Research, 14: 251 (1934).
3. J. Greenspan and S. J. Stein, "A Rapid Colorimetric Method for the Determination of Fluoride Ion Using 'Ferrisal' Reagent," Report of Nov. 9, 1944.

Chapter 3

STATISTICAL METHODS

By D. V. Tiedeman, L. S. Kogan, and M. J. Wantman

The term "statistical analysis" includes many kinds of operations with numerical data. Some of the operations are so thoroughly embedded in ways of working that they seem to be an essential part of collecting data. When these aspects are coupled with the less familiar techniques it is possible to distinguish three major levels of statistical analysis:

1. MAJOR LEVELS OF STATISTICAL ANALYSIS

1.1 Presentation of the Results of an Experiment in Compact Form. This usually involves tabulation and classification of data and the computation of two basic statistical constants: (1) some average or "best" measure of central tendency and (2) some measure of "spread" or "scatter" of an obtained distribution.

1.2 Expression of Relationships in Collected Data. If an experiment has not been affected unduly by extraneous variables, often the data need only be graphed, whereupon relationships are apparent from inspection. If the results are somewhat irregular, some sort of conventional technique is necessary to locate the best-fitting graph. Also in many cases some standard expression for the implied relationship is required.

1.3 Establishment of Probability Values or "Levels of Confidence" for any Statistics Derived from the Investigations. The determination of such probabilities involves computation of the "errors" to be associated with the statistical constants derived from the data and/or tests of significance. The major question to be answered in this stage of analysis is: Are the uniformities, discrepancies, trends, relationships, etc., found in this set of data merely a product of chance sampling, or can it be stated with confidence that they are the result of the experimental procedure? The answer to this question is related to functions of mathematical probability, chief among which is the well-known "normal" or "Gaussian" curve of error.

In this chapter the computing methods at each of the three levels of analysis are reviewed, and the reasons for using these methods are discussed briefly.

(a) Presentation of Results in Compact Form and Computation of Basic Constants. Classification and tabulation of data have been markedly facilitated by use of International Business Machines (IBM) punched-card procedures. For the most part, measures obtained in the various experiments have been punched into standard cards, and a large portion of subsequent treatment has been handled by IBM equipment, namely, the sorter, the collator, the interpreter, the tabulator, and the reproducing summary punch.

Two chief statistical constants have been computed as measures of central tendency. These are (1) the arithmetic mean and (2) the median. Formulas for these statistics are as follows:

Arithmetic mean (A.M.), or $\bar{X} = \frac{\Sigma X}{N}$ (commonly referred to as the "mean")

where X is the value of one item in a sample, N is the number of items in a sample, and Σ denotes summation.

Median, or Mdn = mid-value when measures have been arranged in order of size

In general, the arithmetic mean is the preferred descriptive constant for a set of data, since it is usually the most reliable measure.

Example: Arithmetic Mean. The arithmetic mean of a hematological variable is illustrated as follows:

Table 3.1

Animal no.	Erthyrocytes, millions
1	5.9
2	5.6
3	6.4
4	8.0
5	6.0
	$\Sigma = 31.9$
	$N = 5$
	A.M., or $\bar{X} = 6.38$

The median has been used at times, however, when extreme values are felt to be influencing the size of the arithmetic mean to an excess degree or when limitations of measurement make it impossible to obtain precise values for some of the measures.

Example: Median.

Table 3.2—Urinary Protein Determinations of Rabbits

Data as collected		Protein values arranged in order	
Animal no.	Protein, mg %	Animal no.	Protein, mg %
1	120	2	40
2	40	3	100
3	100	1	120
4	160	7	140 (median)
5	800	4	160
6	250	6	250
7	140	5	800

The basic measure of dispersion used has been the standard deviation. Since the "small-sample" formula is universally applicable, it has been used generally for computation. The following is the formula for the standard deviation:

$$\text{Standard Deviation } (\sigma \text{ or S.D.}) = \sqrt{\frac{\Sigma(X - \bar{X})^2}{N - 1}}$$

This may also be written as

$$\sigma = \sqrt{\frac{\Sigma X^2}{N - 1} - \frac{(\Sigma X)^2}{N(N - 1)}}$$

In all computations the use of "observed-values" formulas rather than those in deviation or "grouped" form has been favored because of the availability of IBM equipment and calculators.

Example: Standard Deviation.

Table 3.3—Weights of Male Dogs

Animal no.	Weight, kg	Animal no.	Weight, kg
23	8.4	30	9.7
24	7.6	31	14.1
26	7.1	36	7.5
28	9.7	37	10.6

$N = 8$

$\Sigma X = 74.7$

$\Sigma X^2 = 734.33$

$N(N-1) = 56$

$N\Sigma X^2 - (\Sigma X)^2 = 294.55$

$\sigma = 2.3$

A.M., or $\bar{X} = 9.3$

In some of the work it has been found necessary to make use of the so-called "weighted mean" and "weighted standard deviation." This is done when the items are felt to be of varying importance in their contribution to the two basic statistical constants.

In the dust-concentration data, the time for which a given concentration extended was not constant. Hence each concentration was weighted by the time of its duration. The formula for the weighted mean is as follows:

$$\text{Weighted Mean} = \frac{\Sigma W_i X_i}{\Sigma W_i}$$

Similarly, the standard deviation for the dust-concentration studies was computed after variables had been weighted by the time factor.

Example: Weighted Mean and Weighted Standard Deviation.

Table 3.4—Means and Standard Deviation of Concentrations

Date	Time elapsed, hr	Weighted mean	Weighted S.D.
4/13/45	5.83	0.673	
4/14/45	2.00	0.972	
4/16/45	5.83	0.739	
4/17/45	5.83	0.849	
4/18/45	5.00	0.669	
4/19/45	5.83	0.781	
4/20/45	5.00	0.716	
4/21/45	2.00	0.818	
4/23/45	6.00	0.662	
4/24/45	5.34	1.042	
4/25/45	5.50	0.626	
4/26/45	4.59	0.698	
4/27/45	5.16	0.769	
4/28/45	2.00	0.702	
4/30/45	5.34	0.711	
5/1/45	5.58	0.658	
5/2/45	5.50	0.635	
5/3/45	4.50	0.874	
5/4/45	5.50	0.649	
5/5/45	2.00	0.582	
5/7/45	5.42	0.910	
5/8/45	5.42	0.578	
5/9/45	5.42	0.582	
5/10/45	5.42	0.723	
5/11/45	5.42	0.698	
5/12/45	1.83	0.978	

Table 3.4 — (Continued)

Date	Time elapsed, hr	Weighted mean	Weighted S.D.
5/14/45	5.50	0.656	
5/15/45	5.58	0.827	
5/16/45	5.50	0.802	
4/13/45 to 5/16/45	139.84	0.735	0.171

(b) Expression of Relationships. The standard measure of relationship used in these studies has been the Pearson product-moment coefficient of correlation. This statistic measures how closely two variables are related as expressed by a straight line. It varies from a value of 0, denoting no relationship, to a value of 1, denoting perfect relationship. The coefficient of correlation is always a signed statistic, a positive sign (+) indicating positive relationship (the higher the one variable, the higher the other member of the pair) and a negative sign (−) indicating inverse relationship (the higher the one variable, the lower the paired variable).

The formula for the correlation coefficient is

$$r_{12} = \frac{\frac{\Sigma X_1 X_2}{N} - \frac{\Sigma X_1}{N} \cdot \frac{\Sigma X_2}{N}}{\sigma_1 \sigma_2}$$

where X_1 is the first variable, and X_2 is the second variable.

Example: Product-Moment Correlation Coefficient.

Table 3.5 — Correlation of Blood NPN with the Ratio of Creatine and Creatinine

X_1 NPN	X_2 Ratio	X_1 NPN	X_2 Ratio
38	.10	71	.16
40	.13	115	.52
38	.11	94	.28
35	.05	104	.28
38	.05	127	.52
36	.07	110	.66
30	.09	280	.88
53	.11	215	.82
33	.03	40	.18
78	.36	42	.16

Table 3.5—(Continued)

X_1 NPN	X_2 Ratio	X_1 NPN	X_2 Ratio
32	.12	42	.05
48	.10	43	.10
37	.04	75	.54
86	.16	220	.10
53	.10	99	.02
41	.09	94	.18
54	.04	36	.12
28	.09	40	.11
42	.09	59	.06
69	.20	33	.03
40	.39	63	.63
77	.07	249	.43
37	.08	38	.22
48	.08	52	.09

$$N = 48$$

$$\Sigma X_1 = 3{,}452$$

$$\Sigma X_2 = 9.89$$

$$\Sigma X_1^2 = 405{,}340$$

$$\Sigma X_2^2 = 4.1767$$

$$\Sigma X_1 X_2 = 1{,}102.58$$

$$r_{12} = .68$$

The product-moment correlation is a measurement of concomitant linear relationship and can also be used for predicting one variable from the other. The equation for using the correlation in predicting one variable from another is known as the regression equation and is as follows:

$$\tilde{X}_2 = r_{12}\frac{\sigma_2}{\sigma_1}X_1 - r_{12}\frac{\sigma_2}{\sigma_1}\bar{X}_1 + \bar{X}_2$$

which can be simplified into

$$\tilde{X}_2 = bX_1 + a$$

where

$$b = r_{12}\frac{\sigma_2}{\sigma_1} \quad \text{and} \quad a = \bar{X}_2 - r_{12}\frac{\sigma_2}{\sigma_1}\bar{X}_1$$

This equation is the familiar one from analytic geometry for a best-fitting straight line.

Example: Regression Equation (Linear).

Relation between colorimetric and gravimetric analyses:

1. The correlation between 83 pairs of colorimetric and gravimetric analyses is .418. The mean colorimetric value for these samples is 0.521 and the standard deviation is 0.171. The mean gravimetric value is 0.729 and the standard deviation is 0.230.

2. The best prediction of colorimetric value ($\tilde{X}_2$) from any particular gravimetric value (X_1) in this experiment can be made from the equation

$$\tilde{X}_2 = 0.311X_1 + 0.294$$

(c) Establishment of Probability Values and Tests of Significance. Practically all the analyses falling into this category have been based on sampling theory as associated with the Gaussian curve of errors and related functions. The basic assumption made is this: If the experiment were to be repeated many times and the same statistic calculated at each repetition, the set of values so obtained would form a normal distribution. Putting this another way: Given a population of measures with a true mean of $\bar{M}$, it might be expected that the means of any samples investigated would be distributed in a symmetrical bell-shaped manner about the population mean.

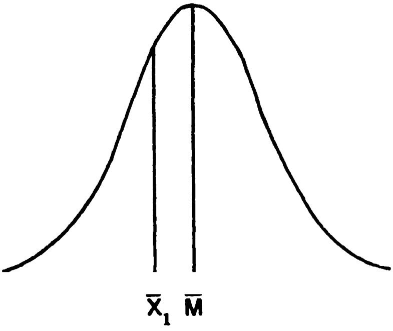

Fig. 3.1—Distribution of sample means.

Thus, in Fig. 3.1, $\bar{M}$ is the population mean and $\bar{X}_1$ is the mean of the sample. By reference to standard tables of the normal probability integral, the P value, or the probability that a deviation as large as the one found is due merely to chance combination of extraneous variables, can be expressed. Since in most cases the true mean and the standard deviation of sample means are not known, they are estimated from the sample. The standard deviation (standard error) of a mean is usually obtained from the formula

$$\sigma_{\overline{X}} = \frac{\sigma}{\sqrt{N}}$$

where σ is the population standard deviation as estimated from the sample and N is the size of the sample. This $\sigma_{\overline{X}}$ tells within what limits the means of other samples of the same size selected from the same parent population are expected to be found. Similarly the standard error of a median is

$$\sigma_{\text{Mdn}} = \frac{1.2533\sigma}{\sqrt{N}}$$

and that of a σ is

$$\sigma_{\sigma} = \frac{\sigma}{\sqrt{2N}}$$

Example: Standard Error of a Mean.

In the previously cited experiment (see Table 3.3) where the mean and standard deviation of the weights of a group of eight dogs were found, the standard error of the mean can be computed as follows:

$$\text{S.E. of Mean} = \frac{\sigma}{\sqrt{N}} = \frac{2.3}{\sqrt{8}} = 0.813$$

This S.E. of the mean can be interpreted in the following manner: If sets of eight animals from the same parent population of animals were selected, the means would fall within the range 9.3±0.813 approximately 68 per cent of the time and within the range 9.3±1.626 about 95 per cent of the time. This interpretation is not strictly precise but serves fairly well for most practical purposes.

Tests of significance of differences are likewise related to the normal curve of error. One of the most frequently used tests in this connection is that applied to the difference between two means (e.g., an experimental- and a control-group mean). The question is put in terms of a "null hypothesis": What are the chances of finding a difference as large as or larger than the one observed if in reality there are no differences between the groups except those due to random fluctuation? Tests of the significance of differences between two means usually fall into one of the following three types:

1. Samples from two independent populations.

$$T = \frac{\bar{X}_1 - \bar{X}_2}{\sigma_d} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\sigma_{\bar{X}_1}^{2} + \sigma_{\bar{X}_2}^{2}}}$$

2. Correlated samples.

$$T = \frac{\bar{X}_1 - \bar{X}_2}{\sigma_d} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\sigma_{\bar{X}_1}^{2} + \sigma_{\bar{X}_2}^{2} - 2r_{12}\,\sigma_{\bar{X}_1}\sigma_{\bar{X}_2}}}$$

3. Independent samples from one parent population.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\dfrac{\Sigma(X_1 - \bar{X}_1)^2 + \Sigma(X_2 - \bar{X}_2)^2}{N_1 + N_2 - 2}}\sqrt{\dfrac{N_1 + N_2}{N_1 N_2}}}$$

The equation for type 1 can be considered to be a special case of type 2 where the correlation between groups is zero. Probability evaluations of (1) and (2) are derived from tables of the normal integral or from a distribution known as "Student's t distribution" when the N is less than 30. However, uses of (1) and (2) are usually limited to large samples, and the use of (3) is more general for small samples. P values for (3) are derived from Student's t distribution. When values of $N_1 + N_2 - 2$ exceed 30 for (3), the P values are found by reference to the normal distribution.

Example: t Test of Significance of Difference.

Table 3.6—Values of t Ratios between Mean Skeletal Uranium Content of Animals on Low- and High-calcium Diet

	N		Mean		σ		$M_A - M_B$	t	P
	A*	B†	A*	B†	A*	B†			
Group I	13	13	92.23	136.92	92.84	84.59	-44.69	-1.28	.20
Group II	11	13	128.91	142.77	89.58	78.91	-13.86	- .40	.69

*A = low-calcium diet.
†B = high-calcium diet.

Group I consists of animals sacrificed 8 days after start of exposure. Group II comprises animals sacrificed 18 days after start of exposure. A difference between means for low-calcium diet and high-calcium diet as large as or larger than the one observed for Group I could have arisen by chance in 20 out of 100 similar repetitions of the experiment, while the difference in Group II means could have arisen by chance in 69 out of 100 repetitions. Hence for these two groups the calcium diet does not seem to have affected skeletal uranium content to any significant extent, even though the difference in means appears fairly large. At least from the evidence in these data there is no valid reason for rejecting the null hypothesis of no difference between the groups. The failure of the difference to be "significant" is largely due to the high variability in the groups.

Another frequently used test of significance is the chi-square (χ^2) test. It has had two main uses in this work: (1) to decide whether there is a significant association between two variables, and (2) to determine whether some theoretical distribution fits the observed data. Applications of chi-square tests have largely been limited to data for which members in a sample can be categorized into mutually exclusive classes, each class or cell containing an observed frequency. The total χ^2 can be found from the equation

$$\chi^2 = \sum \left[\frac{(f_o - f_t)^2}{f_t} \right]$$

in which f_o = observed frequency, and f_t = theoretical or expected frequency. The P values for a given χ^2 are obtained from standard tables of the function. In general, the greater the discrepancies between observed and expected values, the lower the P values obtained. If the theoretical values cannot be determined by hypothesis, they are usually estimated from the observed data themselves according to simple probability considerations based on marginal totals.

Example: Chi-square Test.

Table 3.7 —Genitourinary Damage in Rabbits

	Control	Experimental	Total
Interstitial nephritis	(13.6) 8	(12.4) 18	26
Normal kidney	(10.4) 16	(9.6) 4	20
Total	24	22	46

$\chi^2 = 9.1$ $P = < .01$

In Table 3.7 the control and experimental animals distribute themselves differently with respect to the specific renal disorder under consideration. Each entry is a frequency. The numbers in parentheses are theoretical frequencies and have been determined from the marginal totals; e.g., for the upper left cell the theoretical entry is obtained from $24/46 \times 26/46 \times 46 = 13.6$. The P level of $<.01$ indicates that a X^2 equal to or greater than the one obtained might be expected to occur by chance less than once in 100 trials. Hence it is very likely that the occurrence of interstitial nephritis is significantly greater in the experimental than in the normal animals, or, more broadly, there seems to be an association in that direction.

2. LIMITATIONS OF STATISTICAL ANALYSIS

In all statistical treatment of data there is a risk in assuming that elaborate statistical analysis can somehow supplant proper experimental control. This is never true. All the tests that are applied to the results of these investigations are based on hypotheses about population values. In applying these tests it is assumed that errors of measurement are caused by a large number of uncontrolled factors that do not operate in any consistent or "biased" way.

The limitations of statistical analyses are especially evident even in the simplest of statistical tests, e.g., the difference between two means. In making this test, for example, it is assumed that each mean would be normally distributed about some particular value if the experiment were repeated many times. It is also assumed that irrelevant variables have not affected the measures in such a way that "biased" estimates of population values are made. Finally, the results of the test are stated in a form such as "the difference between these two means or a greater difference might have arisen by chance 5 times out of 100." The experimenter then must decide whether this statement gives him enough confidence to state that the difference is of practical importance or, in this specific case, of medical importance. He runs the risk of denying a real difference, attributing a difference to an experimental condition when the difference may be caused by another uncontrolled factor, or maintaining an insignificant difference as real depending on what P value he is willing to accept as critical. The statistical test, in other words, never proves a hypothesis; it merely renders the hypothesis more or less tenable. The responsibility for "positive" conclusions from any experiment rests solely on the shoulders of the investigator; statistical tests merely facilitate his decisions.

3. SUMMARY

In many of the studies of this volume, data were limited to small samples because of practical considerations. Although the "statistics of small samples" are mathematically just as rigid as those for large samples, it is evident that as much faith cannot be put in the assumptions in small samples, and hence precision and predictive efficiency are likely to be lower.

Standard references for statistical methods are listed below. An attempt has been made to arrange them according to their complexity.

R. Pearl, "Introduction to Medical Biometry and Statistics," W. B. Saunders Company, Philadelphia, 1940.

*G. W. Snedocor, "Statistical Methods," Collegiate Press, Inc., of Iowa State College, Ames, Iowa, 1946.

*C. C. Peters and W. R. Van Voorhis, "Statistical Procedures and Their Mathematical Bases," McGraw-Hill Book Company, Inc., New York, 1940.

*G. U. Yule and M. G. Kendall, "An Introduction to the Theory of Statistics," Charles Griffin & Co., Ltd., London, 1946.

C. H. Goulden, "Methods of Statistical Analysis," John Wiley & Sons, Inc., New York, 1939.

T. L. Kelley, "Fundamental of Statistics," Harvard University Press, Cambridge, Mass., 1947.

*R. A. Fisher, "Statistical Methods for Research Workers," Oliver & Boyd, Ltd., Edinburgh and London, 1944.

*Abbreviated tables of the normal curve, X^2, and t distributions can be found in these volumes. Complete tables appear in the following:

K. Pearson, "Tables for Statisticians and Biometricians," Biometric Laboratory, University College, Cambridge University Press, London, Vol. 1, 1930; Vol. 2, 1914.

T. L. Kelley, "The Kelley Statistical Tables," The Macmillan Company, New York, 1938.

R. A. Fisher and F. Yates, "Statistical Tables for Biological, Agricultural, and Medical Research," Oliver & Boyd, Ltd., Edinburgh and London, 1938.

Chapter 4

THE PATHOLOGICAL ANATOMY OF URANIUM POISONING

By Thomas B. Barnett and Roger G. Metcalf *

1. INTRODUCTION

This chapter deals with the gross and microscopic pathological changes observed in the body tissues of experimental animals administered various compounds of uranium by inhalation, ingestion, injection, and by application to the skin and eyes. It includes a rather detailed descriptive review of the characteristic structural abnormalities associated with uranium poisoning, as well as a discussion and interpretation of these abnormalities. Reference to the literature on the subject of uranium poisoning is limited to such work as is directly related to the interpretation of the observations made in this laboratory.

Although this review is based on the study of animals exposed by all the methods cited above (see Chaps. 6 to 10 for details of exposure), most of the animals examined were subjected to repeated daily exposure to uranium compounds by inhalation or to uranium compounds in the diet for periods of 30 days or less Somewhat fewer animals were given single intraperitoneal injections. Descriptions of progressive changes in the kidney following or during exposure to uranium are derived from a limited number of renal biopsies performed on dogs and from the study of animals sacrificed at serial intervals. Of particular importance in this respect are those rats subjected to various dietary levels of uranyl nitrate and studied by serial sacrifice over a period of 9 months.† This study is especially valuable because

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* Based on reports and work by Roger G. Metcalf, Thomas B. Barnett, George M. Suter, Frank A. Inda, George W. Casarett, Robert P. Kennedy, and M. V. Anders.

† Rats fed diets containing 0.1, 0.5, and 2 per cent uranyl nitrate were sacrificed in groups of three at each level after 1, 2, 3, 5, 7, and 14 days and at intervals of 2 weeks thereafter (see Chap. 7). In all experiments discussed in Chap. 4, uranyl nitrate was used in the form of the hexahydrate.

of the duration of the experimental period and the uniformity of the results in animals at any given level at any one time.

These studies are subject to certain experimental errors, which may be classified briefly as follows: (1) errors that arise from individual differences in the response of different animals, (2) errors that arise from the uncertainty in the rate and amount of absorption when the animals have been exposed by inhalation and ingestion, (3) errors due to the differences in judgment among the staff members of this laboratory who examined the tissues.

Investigation of errors (1) and (2) was not feasible. However, since it was obvious at the start that no two individuals would ever be in complete agreement in their judgment of damage in tissues, the error that might be introduced by factor (3) was investigated with the aid of the Statistics Department of this project. From the results of a representative experiment in which at least two and sometimes three individuals examined the same slides, the agreement among these two or three individuals was so high that it was concluded that any errors arising from this factor were negligible in comparison with the other two factors.

The study of pathology in these animals, although originally expected to aid in determining the relative toxicities of the various levels and methods of exposure, is not considered here in this respect. The reader is referred to Chaps. 6 to 10 for summaries of the pathological findings at the various levels of exposure. The descriptions and interpretations of findings presented in this chapter should serve as reference for these individual summaries.

In addition to the descriptions of the characteristic abnormalities observed in animals exposed to uranium compounds, there is included a brief presentation of the results of an experiment designed to investigate, by histological methods, the problem of acquired tolerance to uranium (see also Chap. 12). These results, although preliminary in nature, are presented here because of their bearing on the interpretation of certain of the findings associated with uranium poisoning.

2. METHODS OF SAMPLING AND PREPARATION OF TISSUES

In most cases small animals were sacrificed by an overdose of ether by inhalation and dogs by an overdose of sodium pentobarbital injected intravenously. After examination of the body and viscera for gross abnormalities, tissue samples approximately 7 mm in thickness were taken from selected organs and fixed in Zenker's formol or in Bouin's fixative. These were embedded in paraffin and sections 7 μ in thickness were cut and stained with Harris's hematoxylin and eosin. In all cases, samples of grossly abnormal tissue were examined microscopically. In some few instances, special procedures were employed for the demonstration of fat and connective tissue and for the study of the central nervous system and peripheral nerves. Bone marrow smears were stained with Wright's stain.

3. PATHOLOGICAL CHANGES IN ANIMALS EXPOSED TO URANIUM

3.1 Microscopic Renal Pathology. Morphological changes occurring in the kidneys of animals exposed to uranium vary with the dosage level, with the length of the period of exposure, and with the time after exposure. In general, the lesion is of one type regardless of the route of administration. Species differences are not marked and are, for the most part, quantitative. In this review the changes observed in rats are presented in detail since this species has been most extensively studied. Changes in the other species are described in so far as they differ from those in rats.

The following descriptions of renal changes are based on the response of animals to many different compounds of uranium, some of which contain potentially toxic substances other than uranium. Of course, there is no direct evidence that the renal changes observed are due to uranium alone. However, the fact that the type of injury and the sequence of events are the same regardless of the uranium compound used seems to be very strong indication that the renal changes observed are characteristic of uranium. It is not intended to imply that such changes are specific for uranium, since it is well known that other agents produce quite similar effects. Whether or not the presence of certain other elements in the uranium compounds enhances the action of uranium cannot be determined from the data available at the present time.

For the purpose of brevity, the following descriptive terms used in a special or limited sense are defined:

Epithelial Degeneration. Nuclear swelling with condensation of chromatin at the nuclear membrane or nuclear pyknosis in cells adherent to the basement membrane. The cytoplasm may be fragmented or homogeneous and eosinophilic (see Fig. 4.2).

Epithelial Necrosis. Chromatolysis and complete loss of cell outline and cell structure, resulting in the reduction of entire tubular segments to masses of pink-staining granular debris (see Fig. 4.3). The term "necrosis" is also used in referring to epithelial cells that have the characteristics of degenerating cells and that have sloughed into the tubular lumen (see Fig. 4.4).

Early or Actively Regenerating Epithelium. Epithelium with rather large pleomorphic cells that show unusually basophilic cytoplasm, some of which contain mitotic figures while others contain large nuclei with vesicular arrangement of chromatin and prominent nucleoli (see Fig. 4.4).

Typically Regenerating Epithelium.* Epithelium similar to the above in staining characteristics of the cytoplasm but more regular in form and with smaller, more hyperchromic nuclei and with no evidence of mitosis. Associated with this type of epithelium, there may be an apparent piling up of cells in the sections of tubules (see Fig. 4.5).

Atypically Regenerating Epithelium. Epithelium made up of flattened cells in which the cytoplasm is basophilic and the nuclei are elongated and hyperchromic. The flattened epithelium gives the tubule the appearance of being dilated, and in some cases there may be actual dilation (see Fig. 4.6).

Activated Epithelium. Epithelial cells in which the cytoplasm is polychromatophilic and the nuclei are enlarged and contain chromatin arranged in much the same way as that in actively regenerating cells. The brush border characteristic of the lining epithelium of the proximal convoluted tubules may still be demonstrable in these "activated" tubules (see Fig. 4.7).

Atrophic Tubules. Tubules that are narrow in over-all diameter, though, by virtue of an irregularly flattened epithelial lining, the lumen may be of almost normal size or even apparently dilated. The cells lining these tubules show normal staining characteristics and the nuclear chromatin arrangement is that of the normal resting epithelial cell (see Fig. 4.8).

Acute Stage of Uranium Poisoning. That phase in which tubular degeneration, necrosis, and regeneration are the predominant findings in the kidney.

Chronic Stage of Uranium Poisoning. That phase in which tubular atrophy, increase in intertubular connective tissue, constriction of glomerular capsules, and narrowing of the cortex are the predominant findings.

(a) The Renal Lesion in Rats. The descriptions of the progressive renal changes in rats, which are presented below, are arbitrarily divided into four grades of severity, from mild (Grade I) to very severe (Grade IV). The abnormalities are localized in the various zones of the kidney cortex shown in Fig. 4.1. Sections of normal rat kidney are shown in Figs. 4.9, 4.10, and 4.11.

Included in the following descriptions of renal damage are the detectable cellular changes associated with uranium poisoning in both the acute and chronic stages as defined above.

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* The terms typical and atypical were used by McNider[1] to differentiate the two types of tubular regeneration occurring after damage by uranium in dogs.

Damage, Grade I. Repeated daily exposure of rats by inhalation to levels of uranium compounds of the order of 0.3 mg of uranium hexafluoride per cubic meter of air (see Chap. 10, Sec. 3.2) results in a very mild renal tubular degeneration followed by sloughing of tubular epithelium and active regeneration at the site of the degenerative changes. These processes are limited to the proximal convoluted tubules in the arcuate zone of the kidney cortex. Such changes are first observable after 3 to 4 days, and, if very slight, persist up to 10 to 14 days, after which time there are no significant morphological changes during the period from the 16th to the 30th day even though exposure continues.

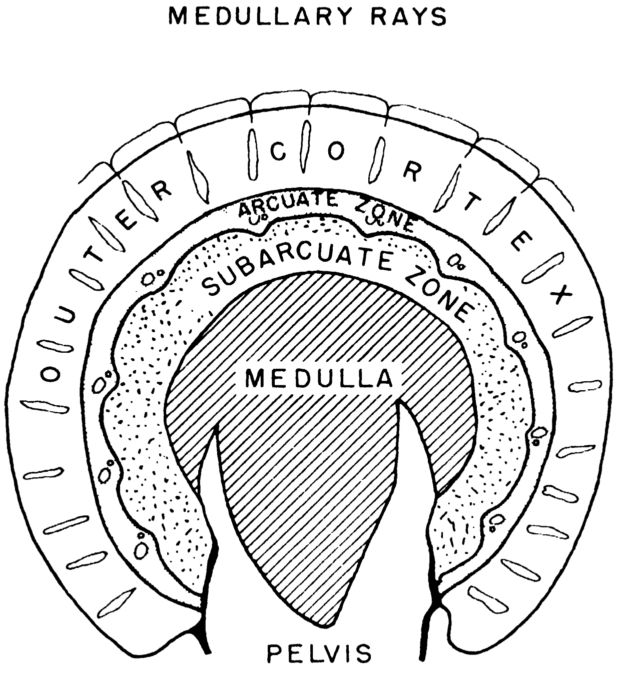

Fig. 4.1—Diagram of a transverse section of a rat kidney showing zones used in description of microscopic renal pathological changes. The arcuate zone is in the region of the arcuate vessels and the deepest row of glomeruli. The subarcuate zone is that commonly referred to as the outer stripe of the outer zone of the medulla.

The occurrence of this degree of damage in animals exposed for periods longer than 30 days has been observed in only one group of rats, namely, those fed a diet containing 0.5 per cent uranyl nitrate hexahydrate (see footnote, Sec. 1). The initial acute damage under these conditions is only slightly more extensive than that described above (see Fig. 4.12), and at the end of 30 days the renal tubular epithelium is essentially normal. After 8 weeks of exposure, however, slight changes, characterized by activation of epithelial cells, are observed rather consistently in the arcuate zone tubules. These cells show polychromatophilic staining of the cytoplasm and an increase in the size of the nuclei with chromatin arrangement similar to that observed in actively regenerating cells (see Fig. 4.13). A few sloughed necrotic cells may also be seen in these tubules. Except for this type of change, no other abnormalities are observed in animals exposed for periods as long as 38 weeks.

Damage, Grade II.* Exposure of rats to somewhat higher concentrations of uranium results in a more extensive and less transient involvement of the renal tubules. Degeneration of tubular epithelium may be seen as early as 24 to 48 hr after exposure starts and at first is limited to segments of the proximal convoluted tubules located in the arcuate zone of the cortex. One to 2 days later, more extensive cell breakdown is observed. Degeneration and necrosis of tubular epithelial cells are seen in the arcuate zone and in the regions just peripheral and just subjacent to it and along the medullary rays. At this same time active epithelial regeneration appears in the mid-subarcuate zone.

By the end of the first week of exposure, the moderately damaged kidney has the appearance shown in Fig. 4.14. Throughout the region just peripheral to the arcuate zone and along the medullary rays there are necrotic tubules in which the basement membrane is stripped of cells and the lumen is occupied by a pink-staining mass of cellular debris (see Fig. 4.3). Here there may be no evidence of epithelial regeneration, although occasionally very flat actively regenerating cells are seen between the necrotic mass and the basement membrane. Deeper in the cortex, in the arcuate zone and just subjacent to it, cellular degeneration and sloughing with associated active regeneration are seen (see Fig. 4.4). Some tubules are completely relined by large, basophilic, bizarre cells, and the lumina contain sloughed necrotic cells. Cells undergoing mitotic division are usually seen with regeneration at this stage. Proximal convoluted tubules still deeper in the subarcuate zone are either morphologically normal or, in some instances, are lined by cells that appear enlarged and contain nuclei resembling those seen in the activated epithelium described in connection with Grade I damage (see Fig. 4.7). These cells are quite regular in shape and mitotic figures are absent. After 1 week there is also an accumulation of hyaline and finely granular casts in the collecting tubules. At this time, there are no changes in the glomeruli that are detectable by the methods used.

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* This description is based principally on the examination of tissues from rats exposed to a diet containing 2 per cent uranyl nitrate hexahydrate (see footnote, Sec. 1) and rats subjected to repeated daily exposure to uranium in the following atmospheric concentrations: 1.9 mg of uranyl nitrate hexahydrate per cubic meter of air, 3 mg of uranium hexafluoride per cubic meter of air, 18 mg of ammonium diuranate per cubic meter of air (see Chap. 10, Secs. 3.2, 3.4, and 3.12). The total number of rats involved in these experiments was 269.

Rats studied at the end of 2 weeks of exposure show rather extensive regenerated tubular epithelium in the zones in which degeneration and necrosis were observed earlier. For the most part, this regenerated epithelium is of the so-called "typical type," although there are usually some tubules lined by a flattened, atypical epithelium. Sloughed necrotic cells and cellular debris are present in many of these tubules, and numerous casts appear in the collecting tubules.

Animals studied during the third and fourth weeks of exposure show renal changes that may vary considerably in different series. In some cases there may be an almost complete return to normal by the end of 4 weeks, as far as renal morphology is concerned, and it is usually possible to demonstrate a greater number of normal proximal convoluted tubules during this period than at any time earlier in the series. The tubular changes that are most commonly demonstrable at this time interval are predominantly regenerative in nature, degenerative changes being limited to a few scattered necrotic cells in the tubular lumina and a few casts in the medullary collecting ducts.

Grade II damage occurred in only two of the experiments in which rats exposed to moderate concentrations of uranium compounds for periods greater than 30 days were studied serially. Moreover, the results obtained from one of these experiments, namely, that in which rats were exposed to inhalation of 1.9 mg of uranyl nitrate per cubic meter of air, show quite marked individual variations. Therefore, these results are of limited value in determining the progressive changes that take place. On the other hand, the experiment in which the rats were fed a diet containing 2 per cent uranyl nitrate hexahydrate show very similar changes in animals sacrificed at the same time. The description that follows is derived principally from the results of this latter experiment.

From the end of the fourth week to the end of the sixth week various changes are noted in the renal epithelium. Typical tubular regeneration is in most cases the predominant feature, although there is usually some atypical epithelium. There is no marked destruction of cells, and the tubules in the areas in which injury was previously observed are lined by cells of one or the other type of regenerated epithelium. There is evidence, however, that the toxic agent is still exerting an effect and that all the changes observed are not merely due to incomplete repair of previous damage. A few sloughed cells are present and a number of tubules appear slightly irregular and are lined by cells which differ from normal only in that they appear activated. It has already been noted that this type of epithelium occurs in the deep subarcuate region as part of the initial acute response. Similar changes are also seen after prolonged exposure to lower concentrations. The significance of this so-called "epithelial activation" will be discussed later.

Animals studied during the period from the eighth to the fourteenth week of exposure show similar changes, except that somewhat fewer sloughed cells are present and tubular atrophy, which may vary considerably in degree in different animals, is noted along the arcuate zone. This tubular atrophy is characterized by a constriction of tubules and a thinning of the epithelial lining, with an apparent increase in intertubular stroma. Associated with this type of change are often seen thin-walled dilated tubules. These changes, when marked, produce an over-all distortion of the renal cortex.

The appearance of the renal tubules is not markedly different in animals examined between 4 and 9 months. There is an increase in the amount of tubular atrophy in the arcuate and subarcuate zones, cellular sloughing is almost negligible, and fewer tubules show activation and typical regeneration. In no case is there a marked interstitial fibrous reaction, although there may be localized areas of fibrous increase, a few round cells, and hyaline thickening of the basement membrane of a few tubules or glomerular capsules. By the end of 9 months there may be some narrowing of the cortex.

After 2 years of exposure, the kidney is likely to show the following abnormalities (see Fig. 4.15): narrowing of the cortex, considerable atrophy and constriction of tubules, with dilation of adjacent tubules, constriction of some glomeruli, mild to moderate interstitial round cell infiltration, and slight localized increase in intertubular stroma. Often associated with this interstitial reaction are tubules and glomerular capsules that show hyaline thickening of basement membrane and hyperplasia of the capsular epithelium (see Fig. 4.16). Irregular localized areas in the cortex show typically regenerated epithelium. It should be mentioned here that in control rats of this age there may be considerable chronic interstitial inflammation and some irregularity in size of tubules and glomeruli.

Rats fed a diet containing 2 per cent uranyl nitrate for periods of 6 to 7 months and then placed on a uranium-free diet for approximately the same length of time show no appreciable renal abnormality (see Fig. 4.17). No serial observations were made so that the progressive events leading to this remarkable recovery are not known.

The tubular changes described above are in the proximal convoluted* tubules. In some instances changes are noted in tubules that are presumably ascending limbs of the loops of Henle, both in the cortex and medulla. The cells lining this segment of the nephron sometimes appear somewhat enlarged, and the cytoplasm takes more of the basic stain than usual.

Damage, Grade III.† During the initial acute phase moderately severe renal tubular damage in rats exposed to uranium does not differ qualitatively from Grade II damage, and the changes are essentially the same, at least during the first 3 weeks, whether the rats are exposed by a single injection, by momentary inhalation, or by repeated daily exposure to atmospheres containing uranium compounds. Degeneration that occurs early (often within the first 24 hr) is quite extensive when first observed, involving proximal convoluted tubules not only in the arcuate zone but in all or nearly all the subarcuate zone and along the medullary rays as well. Many of these tubular segments, and especially those in and near the arcuate zone, are completely necrotic in animals examined 2 to 3 days later. The degree of tubular necrosis is less in the deep subarcuate zone, and although most of the tubules here show degeneration and sloughing of cells, there is not the severe necrosis that occurs in the arcuate zone and there are usually a few cells present that are morphologically normal. As early as 3 days after the beginning of exposure epithelial regeneration is observed. The regenerating cells are enlarged and may be oval, fusiform, or markedly elongated; the nuclei are large and vesicular, and the cytoplasm stains a distinct blue with the hematoxylin-eosin method. These cells appear in marked contrast to the degenerate cells in the same tubules, in which the cytoplasm stains markedly eosinophilic and the nuclei are either very dark and pyknotic or swollen and karyolytic. The actively regenerating cells are more numerous in the cases examined between the fourth and sixth days and, although they are first observed in the deep subarcuate zone, they appear only slightly later and in fewer numbers in the more peripheral zones of damage. Mitotic figures are sometimes quite numerous. By the end of 7 to 9 days, the tubules in the damaged zones are almost completely lined by this basophilic pleomorphic epithelium and the lumina are engorged with sloughed necrotic cells and cellular debris. Hyaline and finely granular casts fill the medullary tubules, and occasionally calcification of these casts occurs. Calcification seems to occur with the greatest frequency in animals exposed by intraperitoneal injection. Accumulation of pink-staining granular debris in the glomerular capsular spaces is a rare occurrence in rats during this stage of injury.

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* It is not always possible to identify postively this segment of tubule when it is severely damaged; however, when this designation is used in this review, it is based on cell morphology, size of the tubule, and location of the tubule in relation to other intact structures in the same section.

† This description is based on the findings in rats treated by the following methods: intraperitoneal injection of 0.5 mg of uranyl acetate per 100 g of body weight; intraperitoneal injection of 0.3 mg of uranyl nitrate per 100 g of body weight; momentary exposure to atmospheric concentrations of uranium hexafluoride of the order of 1,500 mg/cu m of air; repeated daily exposure to uranium hexafluoride in concentrations of approximately 20 mg/cu m of air. Total number of animals in these studies was 154.

Usually by the end of 2 weeks the regenerating epithelium appears less active, and the nuclei as well as the cells are smaller, although the basophilic-staining characteristics of the cytoplasm remain. At this stage the epithelium takes two distinct forms, some of it being the so-called "typical" type and some the flattened, atypical type. The relative amount of each of the two types of epithelium varies in different animals. In general, the more extensive the damage, the greater the amount of atypical epithelium.

By the end of 30 days of exposure, the kidneys of animals surviving the early renal insult are in a remarkable state of repair, considering the extensive acute damage that occurred earlier. There may be seen a few sloughed dead cells in the tubular lumina, but for the most part the kidney is free from active damage and the tubules are lined by the two types of new epithelium. Often the number of completely normal tubules is greater than at any time earlier in the serial study. There may also be a slight apparent increase in intertubular stroma. Chronic interstitial inflammation, constriction and atrophy of tubules, hyaline thickening of the basement membrane of tubules and glomerular capsules, all may be seen in varying degrees at this time. These changes, however, are usually not marked.

As is the case in less severely damaged kidneys, most of the tubular changes occur in the proximal convoluted segment of the nephron. In certain cortical and medullary tubules, which are presumably the ascending portions of the loop of Henle,* changes similar to those described under Grade II damage are occasionally observed during the stage of acute injury. Except for tubular casts and changes associated with interstitial inflammation, no abnormalities are consistently

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* Because of the infrequency of this change and the alteration of the cells, it is not possible to identify definitely this segment of tubule. The localization and morphology of these tubules suggest that they are portions of the ascending limb of Henle's loop.

seen in any other segment of the renal tubular system. Not all the proximal convoluted tubule is injured, as is evidenced by small groups of normal proximal-convoluted-tubule segments remaining in the mid-cortical regions during the period of most extensive damage. Whenever, in these cases, it is possible to observe the neck or connecting segment of the proximal convoluted tubule with the glomerular capsule, the cells in this portion are undamaged.

No serial studies have been made of rats with Grade III damage beyond the 30-day point. A group of 10 rats exposed to uranium hexafluoride in concentrations of the order of 1,500 mg/cu m of air for 10 min and examined 5 ½ months later show kidneys that are essentially normal. This level of exposure results in damage of Grade III severity in the first 10 days following exposure.

Damage, Grade IV. Only a small number of rats examined in this laboratory exhibit damage of greater severity than that described in the preceding section. The few such cases available for study are rats given intraperitoneal injections of 5.0 mg of uranyl nitrate per 100 g of body weight. In such cases it is possible to demonstrate degenerative changes in proximal convoluted tubules in the outer cortex of the kidney within the first 1 ½ hr to 2 hr after the injection. There is progressively more extensive involvement of the cortical tubules in animals studied during the first 48 hr. In one case surviving for 72 hr essentially all proximal convoluted tubules were either necrotic or showed rather advanced degeneration. This case also showed in many remaining tubules (distal convoluted tubules, loops of Henle, and collecting tubules) an epithelial alteration characterized by swelling and basophilic staining of the cells and enlargement of the nuclei resembling somewhat the so-called "activation" previously described. The glomerular capsules contain pink-staining granular material.

(b) The Renal Lesion in Dogs. Although no extensive serial studies of dogs have been carried out, there are available some data from dogs examined either by sacrifice or renal biopsy at various intervals during exposure to uranium compounds by inhalation (see Chap. 10, Sec. 8.4i). In addition, many dogs dying during exposure by inhalation and ingestion or sacrificed after approximately 30 days of such exposure have been studied. The results of these studies indicate that the localization of the renal lesion in dogs is quite similar to that in rats, as are the nature and progression of the acute changes. The differences noted are, for the most part, those which occur in the more chronic stages of the lesion. In dogs the increase in intertubular connective tissue is usually much greater than in rats. This is true of hyaline thickening of the basement membranes of tubules and glomerular capsules also, and, as in rats, there is often a cellular hyperplasia of the capsular epithelium associated with this thickening in the capsules. Interstitial accumulations of chronic inflammatory cells occur more frequently in chronic uranium poisoning in dogs than in rats. In this connection it should be pointed out that spontaneous chronic interstitial nephritis is more common in dogs than in rats (see Table 10.31).

Comparison of the histological appearance of the kidneys of rats and dogs after 30-day exposures by inhalation to the same dosage level of uranium reveals that at this time the over-all abnormalities in dogs are more extensive than in rats. This has been found to be true regardless of the level of exposure. Likewise, in some cases in which the level of exposure produces no damage in rats, mild to moderate damage is apparent in dogs with the same exposure. This has been noted both after 10 days and after 30 days (see Chap. 10, Sec. 11).

(c) The Renal Lesion in Rabbits. The results of a few serial studies of rabbits exposed to uranium by inhalation show very similar changes during the acute phase to those which have been described in rats. Glomerular changes characterized by swelling, hyperemia, and vacuole formation are observed in rabbits during the phase of initial injury (in damage of the order of Grade III) more frequently than in rats. Actual necrosis of the glomerular tuft is a rare occurrence in rabbits following very large doses.

Hemorrhage into the renal tubules and accumulations of acute inflammatory cells in and between the tubules have been observed only in an occasional case. These changes are so rare in occurrence as to constitute an unimportant feature of uranium poisoning produced with the methods used here.

In the more chronic stages of uranium poisoning in rabbits there is usually an increase in interstitial connective tissue and quite often a rather extensive chronic inflammatory cell infiltration. Spontaneous chronic interstitial nephritis is noted as frequently in control rabbits as in control dogs but is usually more extensive in rabbits (see Table 10.31). Interstitial round cells are not always present, however, in rabbits after prolonged exposure to moderately large doses of uranium.

As is the case with dogs, whenever groups of rabbits are compared with rats with the same exposure, the over-all morphological change is greater in rabbits, and at relatively low levels of exposure there may be changes in rabbits when similarly exposed rats are normal. Such comparisons have been possible only after 30 days of exposure by inhalation, since at no other interval are there available sufficiently large numbers of both species that have undergone similar treatment. The marked round-cell infiltration so often seen in the kidneys of rabbits makes the comparison of the two species at the 30-day point even more difficult.

Rabbits allowed to survive 30 days of exposure to a rather severely damaging atmospheric concentration of sodium diuranate and examined at weekly intervals for 12 weeks thereafter show a progressive return to a near-normal morphological condition. Only slight tubular atrophy and distortion remain at the end of 12 weeks (see Chap. 10, Sec. 19).

(d) The Renal Lesion in Other Species. Changes in the kidneys of the few mice and guinea pigs studied in this laboratory are not significantly different from those in rats.

3.2 Gross Renal Pathology. Except in such cases with moderately severe to severe renal damage, obvious alterations in the gross appearance of the kidney are usually lacking in the acute stage. Grossly observable acute damage is characterized by a grayish-yellow zone in the region of the corticomedullary junction of the kidney, the width of which varies considerably. This zone is also characterized by a loss of the normal striated appearance. With higher levels of exposure, this grayish-yellow discoloration extends from the corticomedullary zone in very narrow bands to the surface of the cortex. In such cases, the external surface exhibits a yellowish speckling.

Continuous long-term exposure of rats to relatively high dietary levels of uranium compounds results in a granular kidney surface, narrowing of the cortex, and a kidney that appears smaller than normal in relation to the size of the animal.

Rabbits and dogs exposed to moderately high concentrations of uranium by inhalation and ingestion often show, after a period of approximately 30 days, an actual pitting of the kidney surface (see Sec. 3.1b and c).

3.3 Acquired Tolerance of Renal Epithelium to Uranium Demonstrated Histologically—A Preliminary Study. In animals subjected to repeated daily exposure to uranium compounds by inhalation and ingestion, the peak of cellular destruction occurs during the first 7 to 10 days and, in spite of continued exposure, regeneration occurs and the extensive cell breakdown does not recur. Because of this fact and because of other indications that animals receiving uranium in repeated exposures develop tolerance to the toxic action of uranium (see Chap. 12), an experiment was designed to provide an opportunity to compare the histological renal changes in animals with acquired tolerance with those in control animals following injections of uranium. Control rats and rats previously subjected to diets containing uranium were supplied by Elliott Maynard.

Rats fed diets containing uranyl nitrate in concentrations of 0.1, 0.5, and 2.0 per cent for various time intervals were injected intraperitoneally with 0.3 mg of uranyl nitrate per 100 g of body weight and studied at varying time intervals thereafter. Nonprotein nitrogen (NPN) determinations were done on blood drawn by heart puncture at the time of sacrifice. Control animals of the same age were similarly injected and studied. Similar studies were also made on animals allowed to recover after exposure to a toxic level of uranium. The results are summarized in Table 4.1.

Table 4.1 — Summary of the Results of a Preliminary Study of Acquired Tolerance to Uranium in Rats

No. of animals	Uranyl nitrate in diet, %	Time on diet	Uranyl nitrate injected, mg/100 g of body weight	Findings, 6 days postinjection		
				NPN, mg % Range	Av.	Summary of microscopic renal changes
5	0	1 each at 10, 12, 14, 16 weeks and 2 years	0.3	117–436	216	Moderately severe tubular necrosis in cortex; active regeneration in subarcuate zone (see Fig. 4.18)
5	0.1	1 each at 10, 12, 14, 16 weeks and 2 years	0.3	168–516	324	
5	0.5	1 each at 10, 12, 14, 16 weeks and 2 years	0.3	112–356	210	
6	2.0	6½ months*	0.3	96–408	237	
5	2.0	1 each at 10, 12, 14, 16 weeks and 2 years	0.3	27–68	49	A few necrotic cells in cortical tubular lumina; atrophy and dilation of arcuate and subarcuate zone tubules; many of the latter show activation (see Fig. 4.19)
6	2.0	6 months	0	33–43	38	Tubular atrophy and dilation in arcuate and subarcuate zones

*All rats on 2.0 per cent diet for 6½ months and on uranium-free diet for subsequent 6½-month period prior to injection.

Whether animals are injected after 10, 12, 14, or 16 weeks or 2 years on the diets, the response following the injection of animals from any one diet level is essentially the same. Rats previously unexposed as well as those given diets containing 0.1 and 0.5 per cent uranyl nitrate respond to the injection of uranyl nitrate in much the same way. Six days after the injection the kidneys show rather extensive necrosis of proximal convoluted tubules with active regeneration in the subarcuate zone (see Fig. 4.18).

In contrast to the above response, rats that have been exposed to diets containing 2 per cent uranyl nitrate show changes in the kidney 6 days following the injection (0.3 mg of uranyl nitrate per 100 g of

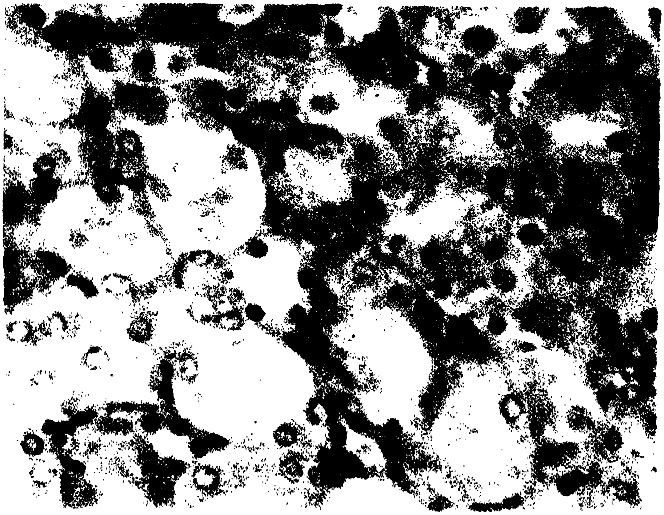

Fig. 4.2 — Tubules in the subarcuate zone of the kidney showing epithelial degeneration. Karyolysis of nuclei and fragmentation of cytoplasm are predominant features. Several tubules in upper right field show condensation of chromatin at the nuclear membrane. A few pyknotic nuclei are shown in the upper field in cells with eosinophilic homogeneous cytoplasm. The three small undamaged tubules are presumably segments of Henle's loop. Rat, 2 days after 10-min exposure to approximately 1,500 mg of uranium hexafluoride per cubic meter of air. 430×

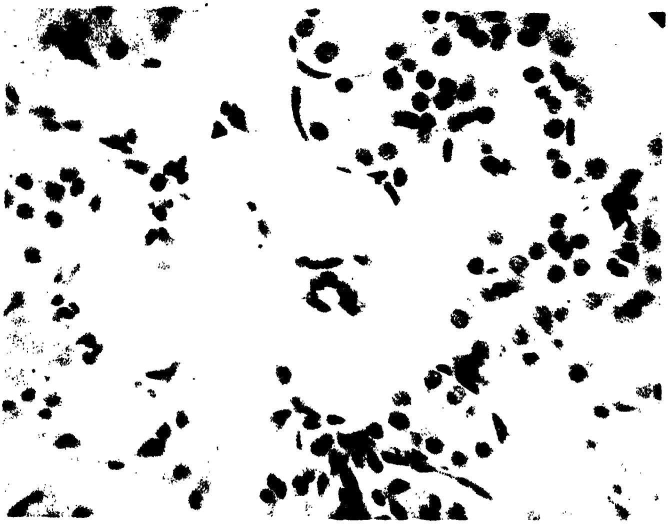

Fig. 4.3 — Tubules in the arcuate zone of the kidney showing complete necrosis of tubular segments. Most of the intact tubules are ascending limbs of Henle's loop and distal convoluted tubules. At the extreme right is an undamaged section of the proximal convoluted tubule. This is presumably from the most proximal part of this division of the nephron. Rat, 4 days after exposure described in Fig. 4.2. 430×

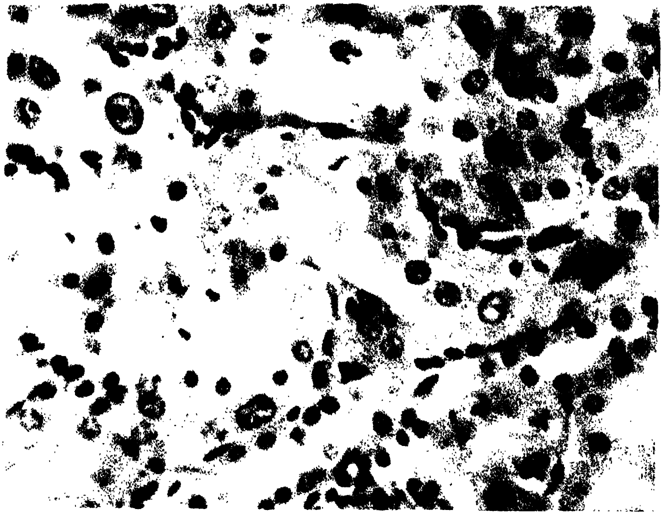

Fig. 4.4—Active epithelial regeneration in tubules in the subarcuate zone of the kidney. Note the large pleomorphic cells with blue or blue-gray cytoplasm, large vesicular nuclei, and prominent nucleoli. Many mitotic figures are present. Degenerate and necrotic cells are also present in these tubules. Rat, after 5-day exposure to 1.9 mg of uranyl nitrate per cubic meter of air. 430×

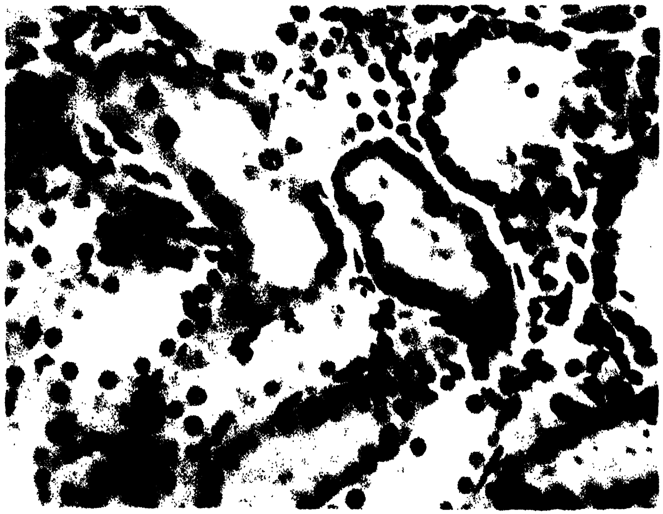

Fig. 4.5—Typical tubular regeneration in tubules in the subarcuate zone. The cells and nuclei are much more uniform in shape than those of Fig. 4.4 although the cytoplasmic staining is similar. The nuclei are nearer the normal size. Note the apparent piling up of cells in the tubules at the right. A few sloughed cells are present. Rat, 4 weeks of repeated daily exposure to 1.9 mg of uranyl nitrate per cubic meter of air. 430×

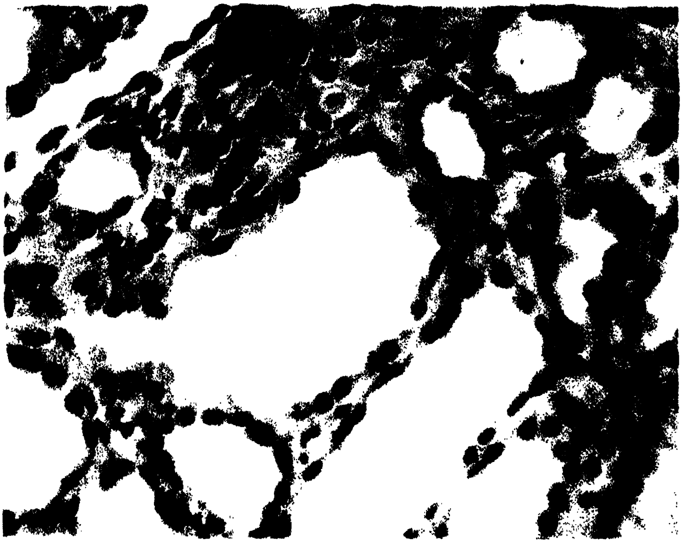

Fig. 4.6—Atypical regeneration in the subarcuate zone of the kidney cortex showing the flattened epithelial lining with elongated hyperchromic nuclei and basophilic cytoplasm. Some eosinophilic amorphous debris is present in the tubular lumina. Rat, 10 days after 10-min exposure to approximately 1,500 mg of uranium hexafluoride per cubic meter of air. 430×

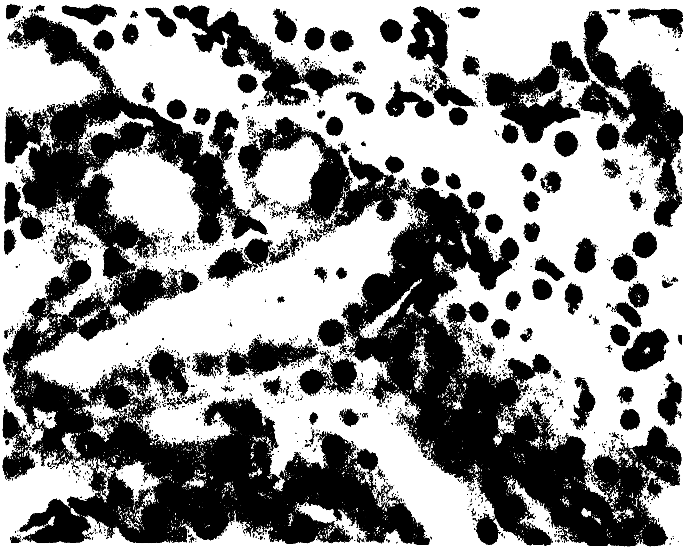

Fig. 4.7—Epithelial activation in tubular cells in the inner subarcuate zone showing large vesicular nuclei and polychromatophilic cytoplasm. Rat, after 1 week of repeated daily exposure to 1.9 mg of uranyl nitrate per cubic meter of air. 430×

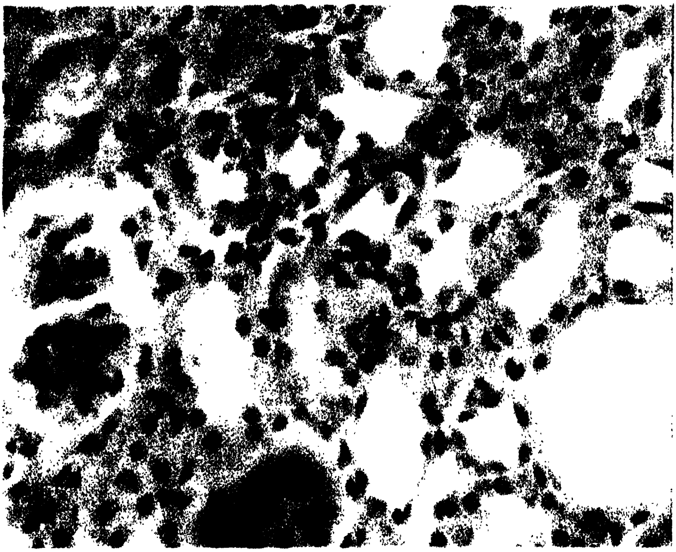

Fig. 4.8—Tubular atrophy in the arcuate zone of the kidney. Note the difference between these thin-walled tubules and those in the same region of the normal kidney cortex (see below). The tubule in the lower right field shows marked dilation. Rat, 12 weeks on a diet containing 2 per cent uranyl nitrate. 430×

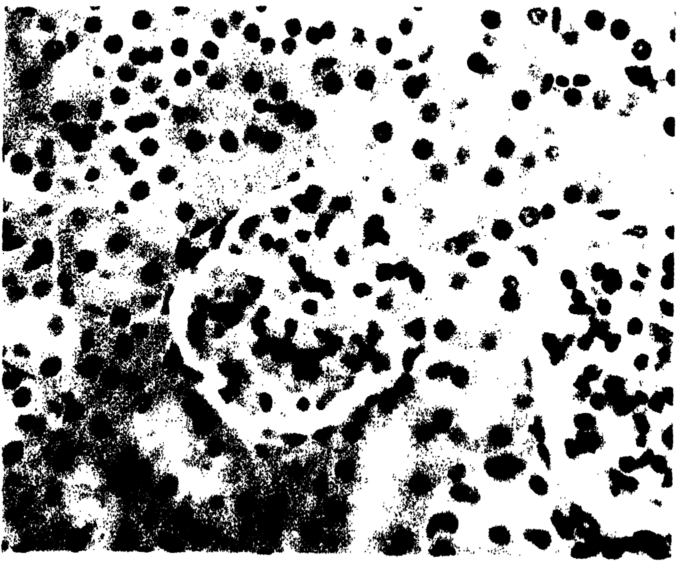

Fig. 4.9—Tubules and glomeruli in the arcuate zone of the renal cortex of a control rat. 430×

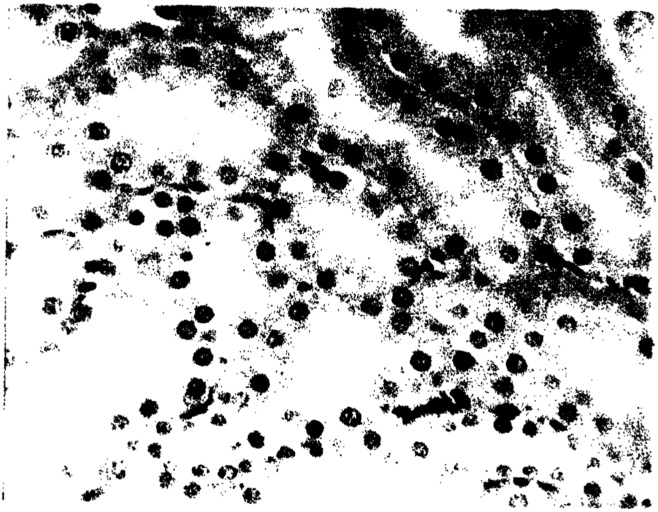

Fig. 4.10—Tubules in the subarcuate zone of the kidney of a control rat. The large tubules with distinct brush borders at the lumen edge of the cells are terminal segments of proximal convoluted tubules. The smaller tubules are segments of Henle's loop. 430×

Fig. 4.11—Renal cortex of control rat. The subarcuate zone is to the left, the outer cortex to the right, and the arcuate zone between. 60×

Fig. 4.12 — Renal damage of Grade I severity in a rat after 1 week on a diet containing 0.5 per cent uranyl nitrate. The tubular degeneration and necrosis and associated active regeneration are limited almost entirely to tubules in the arcuate zone. 60×

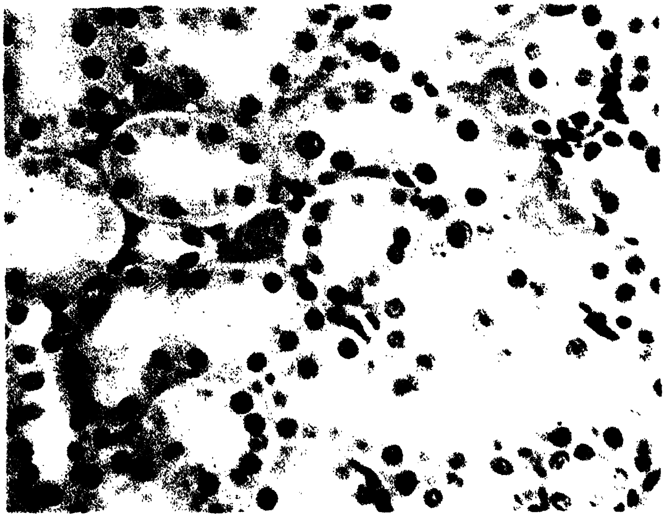

Fig. 4.13 — Proximal convoluted tubules in the outer subarcuate zone of the kidney of a rat fed a diet containing 0.5 per cent uranyl nitrate for 14 weeks. In several tubules there are cells with large vesicular nuclei and polychromatophilic cytoplasm. Two degenerate cells can be seen in this field. 430×

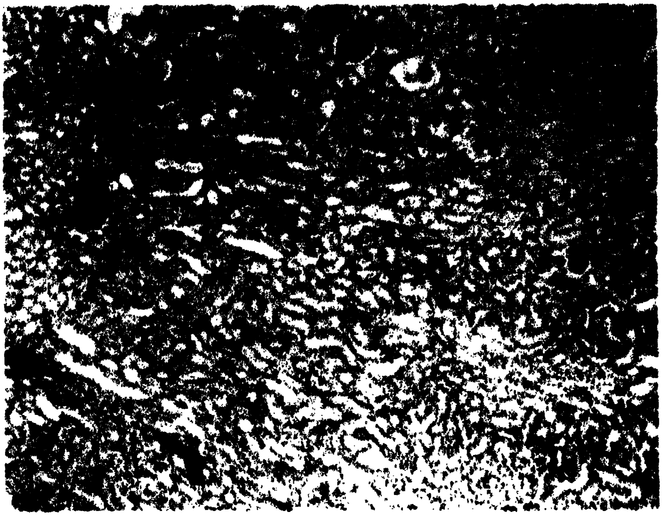

Fig. 4.14—Renal damage of Grade II severity in a rat after 1 week on a diet containing 2 per cent uranyl nitrate. The outer cortex is at the extreme right; the medulla is at the extreme left. Tubular necrosis is most marked in the arcuate zone where there is as yet little regeneration. In the outer subarcuate zone there is tubular regeneration with sloughed necrotic cells in the lumina. Deeper in the subarcuate zone, necrosis is negligible, but the epithelial cells in this zone appear active. 60×

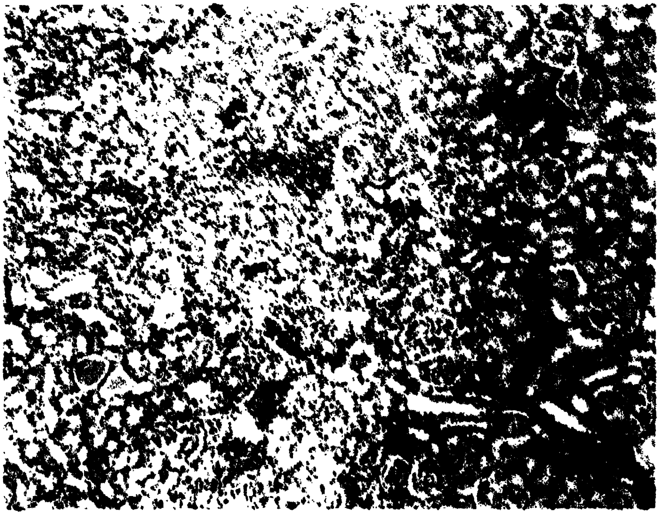

Fig. 4.15—Kidney from a rat fed a diet containing 2 per cent uranyl nitrate for two years. Note tubular atrophy, dilation, and distortion in the arcuate and subarcuate zones. There is moderate chronic inflammatory-cell infiltration (see Fig. 4.16) with a few islands of tubules showing typical regeneration. Only very few sloughed necrotic cells are present. Small calcium deposits like those shown here occur commonly in control rats of this age. 60×

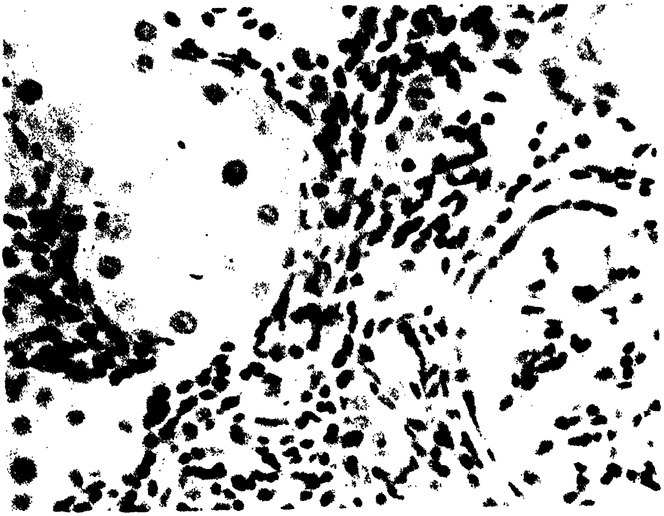

Fig. 4.16—Interstitial reaction of the type present in the kidney shown in Fig. 4.15. There is an increase in interstitial connective tissue as well as an inflammatory-cell infiltration. Note the thickened basement membrane and hyperplasia of the parietal epithelium of the glomerular capsule. 430×

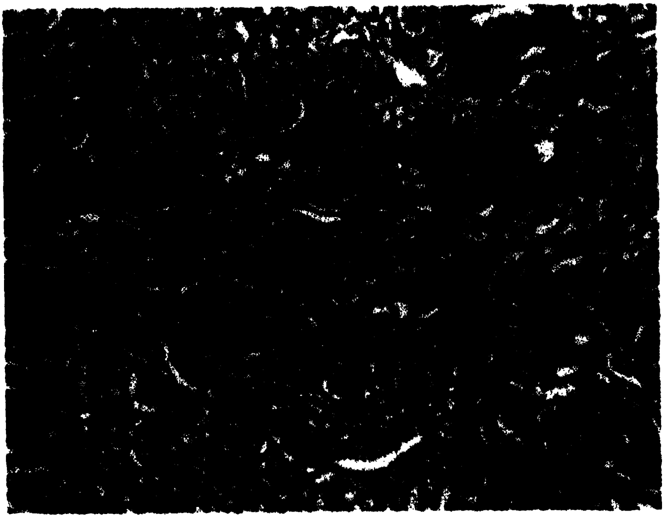

Fig. 4.17—Kidney from a rat fed a diet containing 2 per cent uranyl nitrate for 6½ months and fed a uranium-free diet for a subsequent 6½-month period. This kidney is essentially normal. 60×

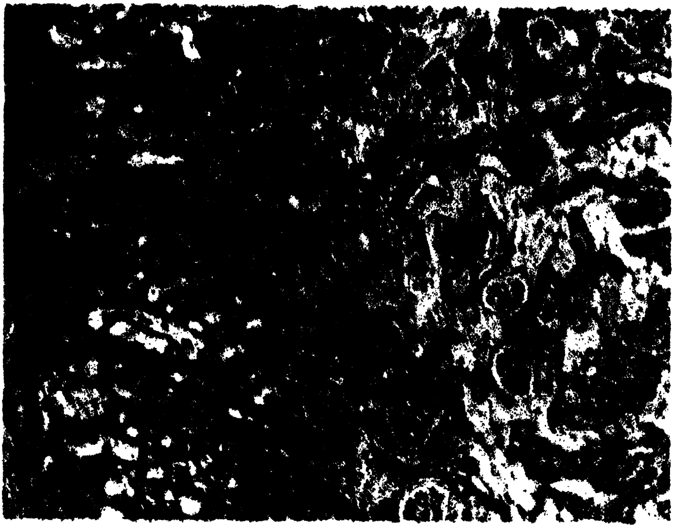

Fig. 4.18—Kidney from a rat sacrificed 6 days after an intraperitoneal injection of 0.3 mg of uranyl nitrate per 100 g of body weight. Prior to the injection this rat had not been exposed to uranium. Note extensive necrosis in the cortical tubules and epithelial regeneration in the subarcuate zone (left). In this case there is amorphous eosinophilic debris in the glomerular capsular spaces and some calcification of casts. 60×

Fig. 4.19—Kidney from a rat sacrificed 6 days after an intraperitoneal injection of 0.3 mg of uranyl nitrate per 100 g of body weight. This rat was on a diet containing 2 per cent uranyl nitrate during the 16 weeks preceding the injection. Note the paucity of cellular degeneration and necrosis. Only very few tubules contain sloughed cells. Note the enlarged nuclei in many of the atrophic tubules. 60×

body weight) which are quite similar to those in rats with the same diet exposure but uninjected. Necrosis of tubular epithelium is limited to necrotic cells in the tubular lumina which are only slightly more numerous than in the rats on the same diet but uninjected. The injected rats also show an apparent stimulation or activation of the cortical tubular epithelium. The constriction, dilation, and distortion of tubules, which result from prolonged exposure to the diet containing 2 per cent uranyl nitrate, are still apparent after the injection; however, the epithelium in these tubules, as well as in other more regular tubules, appears active. The cells and nuclei are larger and the cytoplasm is more basophilic and the nuclei are more vesicular than normal. This epithelium is quite similar to that associated with active regeneration, except that the cells are somewhat less pleomorphic and mitotic figures are rare. This type of alteration occurs primarily in the same zones of the cortex as do the other changes produced by exposure to uranium (see Fig. 4.19).

Essentially the same results were obtained in animals given the same treatment but sacrificed 1, 2, or 3 days after the injection.

Rats exposed to the 2 per cent diet for 6 ½ months and fed a uranium-free diet for a subsequent 6 ½-month period show no detectable evidence of tolerance. The kidney sections are not significantly different from similarly injected but previously unexposed controls. Similarly, rats exposed momentarily to very high concentrations of uranium hexafluoride show no evidence of resistance 5 ½ months later.

In animals in which acquired tolerance is demonstrable histologically, the level of blood nonprotein nitrogen is within normal limits or only slightly elevated 6 days after the injection. This is in contrast to the findings in animals exhibiting no evidence of tolerance, in which moderate to marked increases in nonprotein nitrogen are observed.

3.4 Pathology in Organs Other Than the Kidney. (a) Digestive System. With the exception of an apparently significant increase in observable fat in the livers of rabbits dying during exposure to high atmospheric concentrations (20 mg of uranium trioxide per cubic meter), hepatic changes are observed with significant frequency only in dogs administered uranium in large doses. These changes in the liver are rarely of more than moderate severity, and they take the form of accumulations of fat in the hepatic epithelium or of degeneration and/or necrosis of the epithelium in the centrolobular areas. The relationship between such liver abnormalities and dosage is most apparent in a group of dogs fed diets containing various compounds of uranium (see Table 7.17). The evidence for a similar relationship in dogs exposed by inhalation is rather meager. Such changes are observed in dogs dying during exposure, but the number of such cases examined in this laboratory is very small* (see discussion, Sec. 4.6).

There is no evidence that any of the uranium compounds studied cause damage to the stomach or intestines when administered by injection, inhalation, or application to the skin. When dogs are fed diets containing large quantities of these substances, however, hemorrhage in the mucosa and submucosa of the stomach and intestine occurs rather consistently (see Table 7.17). In some of these cases there may also be hemorrhage and/or ulceration of the buccal mucosa. Similar changes in the oral cavity occur in rabbits exposed to high atmospheric concentrations of uranium tetrachloride (see Chap. 10, Sec. 13).

(b) Respiratory System. When animals are subjected to high atmospheric concentrations of uranium hexafluoride (20 mg/cu m), marked pulmonary hemorrhage and edema occur, with varying degrees of acute inflammatory cell infiltration in the bronchi, alveoli, and alveolar interstices. Although such changes may be seen in rats and dogs, they occur most frequently and with the greatest severity in rabbits. Rabbits also show similar evidence of pulmonary irritation when exposed to lower atmospheric concentration (3.0 mg/cu m). These changes are very similar to those occurring in animals exposed to high concentrations of fluorine and hydrogen fluoride.

Evidence of pulmonary irritation quite like that described above occurs with less frequency in animals exposed to high levels of uranium trioxide, ammonium diuranate, high-grade uranium ore, uranium tetrachloride, uranyl fluoride, and uranium peroxide. As is the case with the hexafluoride, rabbits seem to be much more sensitive to the irritating action of these compounds in the lungs than are rats and dogs.

With few exceptions, animals showing the above pulmonary changes died or were sacrificed within the first week of exposure to these compounds.

(c) Bone Marrow. Bone sections from the distal femoral epiphysis and metaphysis and from lumbar vertebras of experimental animals exposed to uranium compounds show no abnormalities, either in the bone itself or in the marrow, detectable by routine microscopic examination. Bone marrow differential counts were made from smears of femoral marrow from rats exposed to a number of different uranium compounds* by inhalation for approximately 30 days. A comparison of these bone marrow data with those from 109 control rats by the χ^2 technique yields inconclusive results. From the meager data available it can be concluded that the percentage of lymphoid cells in the bone marrow of rats may be increased by exposure to uranium compounds. The data also suggest that myeloblasts may be similarly affected. Both these conclusions must, however, be considered tentative.

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* Two dogs died during exposure to 12.2 mg of uranyl fluoride per cubic meter; two died during exposure to 20 mg of uranium hexafluoride per cubic meter; one was sacrificed when moribund after exposure to 1.9 mg of uranyl nitrate per cubic meter. All these animals showed severe renal damage.

(d) Other Organs. Repeated examination of the spleen, lymph nodes, gonads, heart, skin, adrenal, thyroid, and pancreas from animals exposed to uranium compounds reveals no structural change in any of these organs that can be attributed to the exposure. Careful gross and microscopic examination of the brain stem, selected areas of the cerebral cortex, and selected peripheral nerves (splanchnic and sciatic) from dogs subjected to repeated daily exposure for 30 days to very toxic levels of two uranium compounds by inhalation (20 mg of uranium hexafluoride per cubic meter and 20 mg of uranium trioxide per cubic meter) reveals no structural abnormalities significantly different from those observed in similarly examined control dogs.

4. DISCUSSION OF PATHOLOGICAL CHANGES

4.1 The Effects of Uranium on the Renal Tubule. The preceding review of the findings in experimental animals exposed to uranium compounds points to the renal tubule as the principal and primary site of the toxic action of uranium when administered by the methods and within the dosage range used here. Therefore, in discussing the pathology of uranium poisoning, the localization and progression of the changes in the kidney tubules will first be considered.

(a) Localization of Damage with Respect to the Nephron Unit. In the description of the microscopic appearance of kidneys damaged by uranium, the changes are localized not in specific subdivisions of the proximal convoluted tubule but rather in the proximal tubules in various zones of the kidney (see Fig. 4.1). The subarcuate zone, which is sometimes referred to as the outer stripe of the outer zone of the medulla or, together with the arcuate zone, as the corticomedullary region, is made up of portions of the ascending limb of the loop of Henle and the terminal portions of the proximal convoluted tubules,[2]

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* Uranyl fluoride at 2.5 mg/cu m; uranyl nitrate at 4.5 mg/cu m; uranium dioxide at 22 mg/cu m; uranium trioxide at 20 mg/cu m; uranium peroxide at 19.5 mg/cu m.

Part III of Aschoff,[3] along with a few collecting ducts. According to Maximow and Bloom,[4] the proximal convoluted tubules in the medullary rays are also distal or terminal segments. It is presumed that the segments of the proximal convoluted tubules located in the arcuate zone are, at least for the most part, those of the division just proximal to the terminal portion, i.e., the distal portion of Part II of Aschoff, and that the more proximal segments, Part I and proximal Part II, lie in the middle and outer cortex between the rays.

With this structural basis and with reference to the first part of this chapter, it is possible to localize the various segments of the nephron damaged by uranium. Epithelial cell destruction is observed principally in proximal convoluted tubules, and when the injury is of a very mild degree (Grade I), the damage is limited to that part of the tubule just proximal to the straight terminal portion, i.e., the part in the arcuate zone. The same region is damaged first and most severely with moderately large doses, and in such cases (Grade II) more of the terminal segment, both in the subarcuate zone and along the medullary rays, is involved. With still higher doses the entire terminal segment as well as the more proximal parts are affected (Grade III). In such cases, the only remaining unaffected sections of proximal convoluted tubules are the neck piece, which joins Bowman's capsule, and scattered islands of tubules structurally identical with the neck piece. That this most proximal portion of the proximal convoluted tubule can be damaged is evidenced by the appearance of the kidney in Grade IV injury. After very large injected doses, the earliest epithelial change apparently occurs in a somewhat more proximal segment of the nephron than that showing the first evidence of degeneration with lower doses.

From morphological studies alone the causes for the differences in response of the various segments of the nephron cannot be determined. However, several possible explanations are suggested. Differences in concentration of uranium and/or pH of the tubular urine may play a part, as may differences in the physiological functions of the cells in different segments. That concentration of uranium is important is suggested by the fact that the site of the damage with low doses and earliest damage with moderately large doses is in the distal half of the proximal convoluted tubule, and that the more proximal segments can be affected by increasing the dose. This, however, fails to explain the seemingly greater susceptibility of the distal Part II over the terminal portion, or of the proximal convoluted tubule over the loop of Henle. The latter differences rather suggest that differences in function or specialization of epithelium also play a role. Probably more factors than one are responsible for these differences in sensitivity to uranium.

(b) The Site of Origin of Epithelial Regeneration. The location of the first evidence of regeneration varies with the extent of injury. If the injury is slight and localized to the arcuate segment, regeneration occurs very near the site of the degenerative changes and simultaneously with the sloughing of necrotic cells. The more extensive the tubular injury, the more distal in the tubule does the regenerating epithelium first appear. In this respect, the appearance of the moderately damaged kidney after 1 week is significant. The mid-subarcuate segments of the proximal convoluted tubules show activation and active regeneration with no necrosis, or, at most, a few sloughed cells in the lumen. Slightly more proximal segments show active regeneration and, in the same segments, degeneration and sloughing of cells. In still more superficial areas of the cortex, the tubular segments are completely necrotic and only rarely are regenerating cells present between the necrotic mass and the basement membrane.

This picture suggests that in such cases the source of the new epithelium is the distal undamaged segment of the proximal convoluted tubule, and that these new cells grow up the denuded basement membrane to reline the tubule. It should be kept in mind that cellular destruction is not of equal severity in all segments of the tubule and that the number of spared cells available for regeneration apparently increases from above downward, so that the source of regenerating epithelium may be either the distal uninjured segment or uninjured cells in the more proximal parts, the number of the latter depending upon the degree of damage in any particular segment. Both Hunter[5] and Oliver[6] attribute to slightly damaged, but still viable, cells in the injured segments the ability to supply regenerating epithelium. How much injury a cell can sustain without loss of the ability to proliferate cannot be determined from the present studies. It seems unlikely, however, that regeneration can arise from cells in the regions of extensive necrosis that occur consistently with exposure to large doses. In such cases, the only proximal convoluted tubule cells remaining are those in the extreme distal end of this part of the nephron and in the most proximal division. There is no evidence that repair occurs as a downgrowth from the undamaged neck piece or extreme proximal part of the proximal convoluted tubule. The question of repair arising from cells in Henle's loop is discussed below.

Following the period in which active regeneration is observed, the previously damaged tubules are for the most part relined by new epithelium, which may be either typical or atypical in form. In general, the more extensive the damage, the greater is the proportion of atypical to typical epithelium. The question arises as to the significance of these two types of epithelium. Since typical epithelium is morphologically similar in form and size of cell to normal proximal convoluted tubule epithelium, and since atypical epithelium is flattened and unlike the normal tubular lining, it might be suggested that the former arises from undamaged proximal convoluted tubules, and the latter from the descending loop of Henle, which is lined by a flattened epithelium normally. McNider[7] attributes the difference in type to a difference in source of the regeneration and believes that atypical regeneration arises as an upgrowth from the extreme terminal proximal convoluted tubule cells or the descending limb of Henle's loop. Certainly the present observations support the contention that in severe injury the regeneration arises mainly in the extreme terminal portion of the proximal convoluted tubule. There is no definite evidence that the loop of Henle contributes new epithelium in such cases. Neither can it be proved that such is not the case, although the following observations suggest that it is not: (1) Atypical epithelium occurs in series in which the early damage does not involve the distal half of the subarcuate segment. (2) Even in rather severely damaged kidneys (Grade III), the earliest evidence of regeneration is in the deep subarcuate zone in proximal convoluted tubules. (3) Rats exposed to doses producing severe injury followed by atypical regeneration show a return to morphological normalcy if given sufficient time for repair without further exposure to uranium.

Assuming that part or all of the atypical regeneration arises from the uninjured cells in the extreme distal proximal convoluted tubule, then the morphological difference between typical and atypical epithelium cannot be attributed to differences in the parent cell since the cells in the proximal end of Part III are not markedly different morphologically from those in the distal end.

Although the possibility remains that Henle's loop may supply some new cells, it seems more logical to attribute the morphological characteristics of atypical epithelium to the inability of a few remaining undamaged cells, regardless of location, to reproduce normal epithelium over such an extensive area in such a short time. From the study of rats allowed to recover after a severely damaging dose, there seems to be little doubt that these cells are capable of returning to normal eventually.

(c) Tubular Changes in Animals Subjected to Chronic Exposure. Continuous exposure of rats to very low but mildly damaging dietary levels of uranium (0.5 per cent uranyl nitrate) produces slight cellular destruction that is followed by repair, and after 4 weeks there is a return to almost complete normalcy in spite of continued exposure. At no time later do these acute changes recur, although throughout a period of 9 months there are very subtle changes characterized by an apparent stimulation or activation of epithelial cells and occasional sloughed necrotic cells in the same region in which the initial damage occurred. The kidneys in rats exposed for 30 days to higher levels (2 per cent uranyl nitrate in the diet) show regenerated epithelium of both the typical and atypical types as well as some slight sloughing of necrotic cells and slight activation of epithelium. Whereas these latter changes are the only changes at the lower level, they are overshadowed in most of the rats on the 2 per cent diet by typical and atypical regeneration at first, and later (after 8 weeks) by tubular atrophy, dilation, and distortion.

Assuming that in rats on a diet containing uranium the absorption, distribution, and excretion of the toxic agent continue in a uniform manner and at a uniform rate, the fact that regeneration of epithelium occurs in the face of continued exposure is suggestive of an aoquired tolerance to the toxic action of uranium on the part of the regenerating cell.* If it is further assumed that the activation of epithelium is part of the process of regeneration, then the following hypothesis concerning the changes in the renal epithelium with prolonged exposure to mildly damaging levels seems tenable: Following the stage of acute injury, all the epithelium damageable at this level has been damaged, and regeneration is under way. The return of all these regenerating cells to a normal condition probably does not occur simultaneously; at most, only a few return to normal at a time. As they return to normal, they again become susceptible to damage† and are damaged, not in large numbers, but very few at a time so that in one section of kidney there may be seen extremely few necrotic cells or perhaps none at all. This slight damage results in the regeneration and/or activation of adjacent cells, which revert to a condition in which they resist the toxic action of uranium. By this process a very delicate balance is established between the processes of damage and repair, which accounts for the changes observed as well as the absence of more severe damage.

If, on the other hand, the epithelial activation is considered to result from active stimulation of cells by uranium, then it might be suggested that epithelial resistance to uranium depends on the ability of the cell to respond to the stimulus by activation rather than by destruction.

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*The fact that regeneration of tubular epithelium occurs in essentially the same way in rats subjected to repeated daily exposure to high atmospheric levels of uranium compounds as in rats given a single large dose by injection constitutes additional evidence of the acquired tolerance of regenerating cells.

†See Sec. 3.3 for evidence that tolerance acquired by continuous feeding of uranium is lost when the kidney is allowed to return to a morphologically normal condition.

Whereas the renal epithelium in rats exposed to a diet containing 0.5 per cent uranyl nitrate seems to maintain a balance, kidneys in rats on the 2 per cent diet show a progressive increase in tubular atrophy with what is considered a secondary tubular dilatation.

The data available at present are insufficient to establish the cause for these changes developing as a result of chronic uranium exposure. The small flattened appearance of the epithelium in atrophic tubules is similar in some respects to atypical regeneration; however, atypical regeneration is not a predominant feature of the earlier stages of this degree of damage, whereas tubular atrophy and distortion are marked in the later stages. It might also be suggested that with continuous damage the new epithelium takes an atypical form just as it does when the original damage is extensive. Although this remains a possibility, the small amount of necrosis that occurs with the development of the atrophic change is so slight that it seems unlikely that this is a very important factor.

The importance of connective tissue proliferation as a causative factor in tubular atrophy is difficult to ascertain. Certainly there is at least a slight apparent increase in connective tissue in the region of the tubular changes, but in rats the amount of connective tissue between the tubules is never great. The fact that these tubules return to normal when the animals are placed on a uranium-free diet for a sufficient length of time seems to indicate that connective tissue is not an important factor in tubular atrophy, although this is by no means absolute proof that such is not the case.

At the present time the question of the mechanism of the development of the tubular changes in chronic uranium poisoning remains an open one.

The fact has been noted above that rats fed diets containing uranium for long periods of time never show a recurrence of the extensive tubular necrosis that is observed during the first week. This, in itself, suggests that these animals develop a tolerance to uranium. Further evidence of such an acquired tolerance is obtained when rats that have been administered uranium in the diet for an extended period are injected intraperitoneally with a dose that produces rather severe renal damage in controls. The response of the kidney to the injected dose in animals on the 2 per cent diet is markedly different from that in controls. This difference is manifested both by the absence of marked tubular necrosis and by the absence of an abnormal nonprotein nitrogen retention in the animals on the 2 per cent diet. The response in animals previously fed lower dietary levels (0.1 and 0.5 per cent) is not significantly different from that in previously unexposed animals. This does not preclude the possibility that a lower order of tolerance might not have been demonstrated in these animals if a lower or different testing dose had been used or if methods other than microscopic examination of the kidney had been employed.

The acquired tolerance to uranium is apparently lost, at least to a considerable degree, when rats are allowed to return to a condition in which the renal epithelium is morphologically normal. It would seem, therefore, that the above tolerance depends on the development, in the kidney or elsewhere, of physiological changes that are concurrent with the atrophic changes in the renal tubules. If it is again assumed that absorption, distribution, and excretion of injected uranium progresses in the same way in rats previously exposed as in unexposed rats, the condition of tolerance can be attributed to changes in the renal epithelium. The apparent activation of tubular epithelium and the mild desquamation of cells in animals exhibiting tolerance indicate that the injected uranium has an effect on the tubules, but these changes shed little light on the mechanism of the development of tolerance. The suggested role of the atrophic tubules as well as the regenerating epithelium in the development of tolerance seems to be worthy of further investigation.

4.2 Glomerular Changes in Uranium Poisoning. Glomerular abnormalities detectable by the methods used here are so infrequent in occurrence that it is not possible to describe any sequence or progression of glomerular changes. Occasionally, transient swelling and hyperemia are observed early after exposure to high doses of uranium, and in some few cases there is actual necrosis of the glomerular tuft. This latter change is a rare occurrence. Other abnormalities that are in some cases associated with rather severe acute uranium poisoning are a peculiar vacuole formation in the glomerular tuft and accumulations of pink-staining granular material in the capsular space. The significance of these changes is not known.

In the chronic stage of uranium poisoning no changes are observed in the glomerular tuft itself. Changes in the lining of Bowman's capsule, however, may occur rather frequently. These are characterized by thickening of the basement membrane and hyperplasia of the parietal capsular epithelium and are almost invariably associated with an increase in interstitial connective tissue, which suggests that they may be secondary to the interstitial response.

Hyaline bodies in the glomerular tuft, reported by Christian[8] following injection of uranium in rabbits, have not been observed. In this respect, it should be kept in mind that no special staining procedures have been used to demonstrate these changes.

4.3 Interstitial Tissue of the Kidney in Uranium Poisoning. An increase in intertubular stroma with prolonged exposure to uranium has already been mentioned. It is not possible, from the work done here, to establish the mechanism of the development of this increase, nor is there conclusive evidence to support either of the two controversial contentions regarding the relationship between tubular and glomerular constriction and interstitial fibrosis, namely, as to whether the fibrosis results from the direct action of uranium[6] or whether the increase in stroma is secondary to the tubular atrophy.[3]

Interstitial accumulations of chronic inflammatory cells occur frequently enough after prolonged exposure to uranium compounds to suggest at least an indirect relationship between this response and the exposure to these compounds. Since they are not invariably present in association with uranium poisoning and since they occur more frequently and more extensively in species that develop spontaneous chronic interstitial nephritis most readily, this relationship is probably one in which a spontaneous lesion is enhanced by a toxic agent.

4.4 The Question of Renal Recovery Following and During Exposure to Uranium. There seems little doubt that rats surviving the acute effects of a momentary exposure to a high atmospheric concentration of uranium will, if given sufficient time, return to a state in which the kidney is morphologically indistinguishable from that of an untreated animal of the same age. It also seems fairly certain that, following moderately severe chronic damage from exposure by ingestion for as long as 6 to 7 months, the kidney will return to normal if the animal is free from exposure for a sufficiently long period of time. Because only a few such studies have been made, the minimum time required for this recovery is not known. There is an indication (see Sec. 3.1c) that rabbits surviving 30 days of repeated exposure to a rather high atmospheric concentration return to quite near normal in approximately 3 months.

In one study in which uranium compounds were applied to the skin and subcutaneously, the animals dying as a result of this treatment showed severe renal pathology; most of those surviving for as long as 30 days and sacrificed at this time were normal (see Chap. 8). At one time, these results were thought to indicate complete repair within 30 days following severe injury. The fact that these animals did not die is evidence that they either were more resistant or were exposed to lower doses than those dying after treatment. Unless there is evidence, either chemical or clinical, that these particular animals were severely poisoned by uranium shortly after exposure, the fact that they were normal after 30 days must be considered indicative of unusual resistance or failure to absorb sufficiently the toxic substance rather than evidence of complete repair of severe damage.

When, during continuous exposure to uranium by inhalation or ingestion, the level of exposure is low and the initial damage consequently very slight, the results indicate that the kidneys return to very near normal by the end of the fourth week. However, in one such experiment, animals being exposed to a mildly damaging dietary level show slight but definite changes throughout the period from 3 to 9 months (see below). In animals subjected to moderately damaging levels by inhalation and ingestion for prolonged periods of time, there is very definitely a return toward normal in the kidney following the stage of acute injury. The results of certain experiments in which animals were exposed to dusty atmospheres containing uranium compounds suggest that there is a complete return to normal. Considering the possibilities of error associated with a study by serial sacrifice of animals and the possibilities for variations in exposure in any one group, the above-mentioned cases cannot be interpreted as definite evidence of a return to normal in animals during the period of exposure. Because of the possibilities of error and in the light of more recent studies, especially those in which several animals were examined at each interval in the series, the results of the studies done in this laboratory favor the contention that, with the possible exception of very low levels, continuous uniform exposure to uranium does not allow a complete return to a condition of absolute morphological normalcy even though epithelial regeneration does occur in the face of continued exposure.

The question might be raised as to the possibility of a cyclic sequence of events resulting from damage followed by repair, which, in turn, is again followed by damage. In at least one serial study, acute changes occurred early, and later in the series more severe acute damage was observed. In the vast majority of studies, however, the results have been incompatible with this concept of recurring acute damage. The only evidence supporting any type of cyclic response was derived from the two ingestion experiments in which rats were examined in series for 9 months. The findings here do not suggest cyclic changes except in so far as the initial damage is followed by repair which is, in turn, followed by a subtle but definite change quite different from the acute change. These have been described previously and need not be further discussed here.

4.5 Review of the Suggested Sequence of Events in the Kidney in Rats Poisoned by Uranium. Degeneration of proximal convoluted tubule epithelium occurs from a few hours to 3 days following the initial exposure, depending upon the dose used. As has been pointed out, this may involve only the region of the junction of Parts II and III of Aschoff or it may extend over the entire length of the proximal tubule. If the damage is slight, the degeneration is followed in a few days by sloughing of the dead cells into the lumen; if it is severe, large segments of the tubule may be converted into structureless masses of necrotic debris.

Active regeneration begins as early as 1 day after the first degenerative changes appear, and through active proliferation of cells the tubules are relined during the first 2 weeks.

The sloughed cells and necrotic debris pass distally and accumulate as casts in the more distal segments of the nephron. Casts are usually present in greatest numbers at the end of the first week, and at this time, in severely damaged kidneys, they may be extremely numerous in the medullary tubules. The number of casts decreases during the second week, and depending upon the severity, are almost entirely gone by the end of the third or fourth week.

At the end of the third or fourth week, the tubules are for the most part relined by new but less active epithelium, and evidence of cell destruction is limited to occasional sloughed cells in the cortex and a few casts in the collecting tubules. The new epithelium may be either typical or atypical. In general, the more extensive the cellular destruction the greater is the proportion of atypical to typical epithelium.

Presumably, if exposure is discontinued at this time the kidney returns to normal. If exposure continues and the concentration is low, there is slight activation of epithelium and very slight sloughing of cells. In the case of moderate concentrations, there is, in addition, a gradual atrophy of tubules, dilation of tubules, and an over-all narrowing and distortion of the cortex.

4.6 Discussion of Findings in Organs Other Than the Kidney. Whereas the most characteristic morphological manifestation of the toxic effect of uranium is the injury to the renal tubule, under certain conditions of exposure other organs may show evidence of damage.

(a) Liver. The frequency of hepatic changes in animals exposed to uranium compounds under the conditions used here is rarely of sufficient magnitude to even suggest an effect on this organ. In dogs that are given diets containing various compounds of uranium, however, the frequent occurrence of degenerative changes in those animals fed the higher dietary levels and the absence of such changes at the lower levels and in controls seem to indicate that, with sufficiently high doses, liver damage may result from exposure to uranium. It might also be suggested by these findings that the route of administration; as well as the size of the dose, is an important factor where liver damage is concerned. Such an interpretation is clearly unwarranted when it is considered that dogs dying during exposure by inhalation also show hepatic damage. The number of the latter is so small in relation to the number of dogs exposed that the findings are significant only in the light of more frequent occurrence of similar changes in the dogs given uranium in the diet. Although the hepatic changes observed are varied and nonspecific in nature and although their occurrence is limited to animals dying during exposure, the findings indicate that high-dosage exposure to uranium compounds may produce, either directly or indirectly, damage to the liver that is detectable histologically. These findings are similar to those reported by McNider[9] in dogs given rather large subcutaneous doses of uranyl nitrate.

The absence of morphological changes in the liver in most cases of uranium poisoning does not preclude the existence of changes detectable by methods other than histological examination (see Chap. 5).

(b) Gastrointestinal System. Changes in the gastrointestinal tract that can be related to the administration of uranium compounds are limited to the mucosal and submucosal hemorrhage in dogs fed large doses (see Table 7.17). Although these changes are quite varied in severity and localization in the levels of the alimentary canal, there seems little doubt that they are due in some way to the uranium compounds in the diet. The fact that they are limited to animals fed these compounds suggests a local irritative action; however, it may be significant that, with few exceptions, these changes occur in animals with evidence of liver injury.

(c) Lung. There is no evidence that any of the compounds of uranium studied produce an effect in the lung when administered by ingestion or intraperitoneal injection. The response in the lung when animals are exposed to these compounds by inhalation varies considerably with different compounds and in different species, as well as with the concentration used.

Severe pulmonary damage occurs most consistently in animals exposed to uranium hexafluoride, and when the concentration is relatively high (20 mg/cu m), dogs and rats, as well as rabbits, are affected. At a lower concentration (3 mg/cu m), only rabbits show these changes. The changes occurring during exposure to this compound are quite similar to those observed when animals are exposed to high atmospheric concentrations of fluorine and hydrogen fluoride. The fact that the latter is an important decomposition product of uranium hexafluoride presumably accounts, at least in part, for the pulmonary effects of this particular uranium compound (see Chap. 10, Sec. 10).

Uranium tetrachloride, ammonium diuranate, and uranyl fluoride, which also contain elements and radicals other than uranium that may form pulmonary irritants, apparently produce pulmonary damage in rabbits, when this species is exposed to high atmospheric concentrations (see Sec. 3.4b). Uranium trioxide, uranium peroxide, and high-grade uranium ore also seem to be irritating to the lungs of rabbits when administered in high atmospheric concentrations. The significance of these findings, which are limited almost entirely to rabbits dying during the first 2 weeks of exposure, is not clear. They point to the marked susceptibility of rabbits to pulmonary irritation and indicate that certain uranium compounds are irritating to the lung, but the properties of these compounds responsible for this action cannot be determined.

(d) Bone Marrow. In the absence of significant changes in the peripheral blood and because it is not known whether the increased percentage of the two marrow elements (lymphoid cells and myeloblasts) is an absolute or relative increase, the minor changes observed in the bone marrow of animals exposed to certain uranium compounds lack a satisfactory explanation.

5. SUMMARY

The principal pathological change in experimental animals administered uranium compounds is necrosis of the renal tubular epithelium involving predominantly the proximal convoluted tubule. Evidence is offered suggesting that the region of the junction of the middle and distal thirds of the proximal convoluted segment is damaged exclusively with low doses and first and most severely with higher doses, except in the case of large injected doses where the first damage apparently occurs more proximally.

Degenerative changes first appear as early as a few hours after very large injected doses or as late as 2 to 4 days with moderate and small doses by inhalation and ingestion. The peak of epithelial necrosis is apparently reached within from 3 to 6 days following the initial exposure.

In animals surviving the initial injury, epithelial regeneration is observed within 1 to 2 days after the appearance of degenerative changes. There are two types of regenerated epithelium, one of which approaches the normal in form while the other is flattened and atypical. In general, the latter occurs following rather severe damage. There is presented a discussion of the possible significance of these types of epithelium as regards their origin in the nephron.

Injury in the acute phase, comprising both degenerative and regenerative processes, produces the greatest over-all change in the kidney in the period from the fifth to the tenth day following the initial exposure. This acute phase has not been observed to recur in animals subjected to repeated daily exposure by inhalation or to diets containing uranium compounds. Evidence is offered which suggests that there may be a return to a normal morphological condition following rather severe renal tubular injury in rats that are not reexposed.

The changes in the chronic phase of uranium poisoning, as observed in rats fed uranyl nitrate as 2 per cent of the diet for varying periods up to 2 years, are tubular atrophy, dilation, and distortion with an apparent slight increase in intertubular connective tissue and mild interstitial accumulation of chronic inflammatory cells. Such interstitial changes are also frequently observed in dogs and rabbits exposed to uranium compounds for 30 days.

The results of a preliminary study of acquired tolerance to uranium in rats are presented. These results indicate that prolonged exposure to uranium may produce conditions under which injection of an ordinarily damaging dose of uranium results in no appreciable renal tubular necrosis.

Under the conditions of exposure employed, glomerular changes are observed infrequently. Hyperemia and swelling occur occasionally and glomerular necrosis very rarely during the acute stage of uranium poisoning following very high dosage exposure.

Pathological changes in organs other than the kidney as a result of uranium exposure are as follows: (1) degenerative changes in the liver cord cells of dogs dying during exposure to high dietary and atmospheric concentrations; (2) mucosal and submucosal hemorrhages in the gastrointestinal tract of dogs fed large doses of uranium compounds; (3) pulmonary hemorrhage and edema in rabbits dying during exposure to high atmospheric concentrations of certain uranium compounds.

Severe pulmonary irritation in animals exposed to high atmospheric concentrations of uranium hexafluoride is attributable, at least in part, to one of its decomposition products, hydrogen fluoride.

BIBLIOGRAPHY

1. W. deB. McNider, J. Exptl. Med., 49: 387-410 (1929).
2. G. Carl Huber, Renal Tubules, in E. V. Cowdry, "Special Cytology," 2d ed., Vol. 2, pp. 967-968, Paul B. Hoeber, Inc., Harper & Brothers, New York, 1932.
3. L. Aschoff, Arch. Internal Med., 12: 723-738 (1913).
4. A. A. Maximow and W. Bloom, "A Textbook of Histology," 4th ed., p. 492, W. B. Saunders Company, Philadelphia, 1943.
5. W. C. Hunter, Ann. Internal Med., 1: 747-789 (1928).
6. J. Oliver, J. Exptl. Med., 21: 425-451 (1915).
7. W. deB. McNider, J. Exptl. Med., 49: 411-434 (1929).
8. H. A. Christian, Boston Med. Surg. J., 159: 8-9 (1908).
9. W. deB. McNider, Proc. Soc. Exptl. Biol. Med., 16: 82 (1919).

Chapter 5

CHARACTERISTICS OF URANIUM POISONING

By J. Henry Wills*

PART A. DIAGNOSTIC SIGNS AND TESTS

According to Kobert[1] uranium is the most poisonous of all metals when injected intravenously or subcutaneously. When uranium compounds are given by mouth, the water-soluble salts are the most toxic forms of the metal, but such water-insoluble compounds as uranium oxide and sodium uranate are appreciably toxic because they are fairly soluble in dilute hydrochloric acid.[2] When uranium compounds are applied to the other surfaces of the body, the water-soluble salts appear to be the most toxic. The salts containing anions of strong acids tend to be corrosive.

1. LOCAL EFFECTS

1.1 Skin. Uranyl nitrate and fluoride, sodium and ammonium uranates, uranium tetrachloride and pentachloride, uranium tetrafluoride, and various uranium oxides were applied to the bared skin of animals. Uranyl nitrate, uranium pentachloride, and uranium tetrachloride were the only compounds found to produce significant effects, grading from coagulation necrosis to mild erythema. Details of this work will be found in Chap. 8.

1.2 Orbital Structures. The effects of the compounds mentioned in Sec. 1.1 upon the eye and conjunctiva were compared with those of finely powdered talcum. It was found that uranium pentachloride, uranyl nitrate, uranium tetrachloride, uranyl fluoride, sodium uranate, uranium tetrafluoride, and ammonium uranate produced irritative phenomena ranging from ulceration into the anterior chamber of the eye to moderate corneal and conjunctival injection. These effects are discussed in detail in Chap. 9.

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*Work done by J. Henry Wills, Edna R. Main, and Dan L. Adler.

1.3 Respiratory Tract. In animals inhaling dusty atmospheres containing uranyl nitrate or fluoride, uranium hexachloride or tetrachloride, various uranium oxides, or high-grade ore (largely U_3O_8 and UO_2, containing about 58 per cent uranium), significant changes in the trachea and lungs were caused by uranium tetrachloride alone. The respiratory effects will be discussed in Chap. 10.

1.4 Gastrointestinal Tract. Woroschilsky[3] reported that ingestion of uranyl nitrate or of sodium uranium tartrate produced punctiform ecchymoses of the mucous membranes of the pylorus and intestine. However, since similar hemorrhages were found in the mucosa of the bladder and since both Woroschilsky and Jackson[4] found intestinal ecchymoses after intravenous injection of uranium compounds, this effect probably is not dependent upon local concentration of uranium on the mucosal surface. During the present investigation, uranyl acetate and nitrate, uranium tetrachloride and tetrafluoride, and three uranium oxides were fed to rats at levels up to 20 per cent of the diet without apparent direct deleterious effect on the gut. These experiments will be reported in detail in Chap. 7.

Uranium tetrachloride produced ulceration and acute irritative changes in the tongue and gingivae of animals breathing an atmosphere containing a dust of this compound, the material probably having entered the mouth by the animal's licking its fur.

2. SYSTEMIC ACTIONS

2.1 Acute Poisoning. Both Woroschilsky[3] and Jackson[4] have reported that following subcutaneous or intravenous injection of large, rapidly fatal (2 hr) doses of uranium compounds there was a marked rise (sodium uranium tartrate) or fall of blood pressure (uranyl acetate or nitrate). Death was due to respiratory paralysis, apparently of central origin.

2.2 Subacute and Chronic Poisoning. (a) Uranium in the Blood. Having gained entrance to the blood stream either by absorption from a body surface or by injection, uranyl uranium is characterized by rapid removal from the blood, comparatively large excretion in the urine, and deposition in the kidneys and bones. Tetravalent uranium is removed from the blood more slowly than hexavalent, is excreted in the urine to the extent of only about one-ninth the excretion of uranyl uranium, and is deposited in the kidney and skeleton to a considerably smaller extent than uranyl uranium. The liver may take up fifteen times as much tetravalent uranium as it does of the hexavalent. The disposition of uranium in the body will be treated in Chap. 11.

(b) Excretion of Uranium. Excretion of uranium is mainly through the kidneys. The excretion of an injected dose of uranyl uranium is rapid; about 20 per cent of the uranium injected intravenously into a rabbit anesthetized with nembutal and infused with saline at a constant rate of 1 ml/min was excreted in the first 3 hr after the injection (Table 5.1). The concentration of uranium in the urine is comparatively high immediately after the injection, but falls to about one-quarter of the peak value within 3 hr. Table 5.1 shows that urinary acidity decreased markedly the excretion of uranyl uranium in the urine, whereas alkalinization of the urine might increase it. The presence of anionic complexing radicals like citrate, lactate, and bicarbonate also promoted excretion in the urine.

Table 5.1 — Urinary Excretion of Uranium in the First 3 Hr after Intravenous Injection of 3 Mg of Uranyl Acetate per Kilogram into Rabbits Infused with Various Solutions *

Condition of rabbit	Infusion	No. of rabbits	Urine pH	Urinary U excretion, % of dose
Oat-fed	Saline	2	4.23	7.10
Normal	Saline	4	7.63	20.98
Normal	1.0% sodium citrate	2	7.65	38.58
Normal	0.87% $NaHCO_3$	2	8.03	80.79
Normal	1.2% sodium lactate	2	8.26	46.73

* Uranium analyses in this and in the following three tables were performed by Robert Fleming and his associates, using the fluorophotometric method.

Tetravalent uranium is excreted in the urine at a much lower level than the uranyl form, but its excretion is somewhat more uniform than that of the uranyl uranium; 3 hr after injection the rate is still about 40 per cent of the peak value. Table 5.2 shows that the presence

Table 5.2 — Urinary Excretion of Uranium in the First 3 Hr after Intravenous Injection of 3 Mg of Reduced Uranyl Acetate per Kilogram into Rabbits Infused with Various Solutions

Condition of rabbit	Infusion	No. of rabbits	Urine pH	Urinary U excretion, % of dose
Normal	Saline	5	7.59	2.41
Normal	1.0% sodium citrate	3	7.81	5.34
Normal	0.87% $NaHCO_3$	3	7.93	4.27

of complexing anions (citrate and bicarbonate) increases the excretion of tetravalent uranium but not to so great an extent as that of hexavalent uranium. It is apparent also from Tables 5.1 and 5.2 that bicarbonate is much more effective as a complexing agent for hexavalent uranium than citrate or lactate, whereas citrate is somewhat more effective as a complexing agent for tetravalent uranium than bicarbonate.

In a series of infused rabbits in which simultaneous clearances of inulin and uranyl uranium were determined, it was found that the clearance of uranium in percentage of the inulin clearance increased markedly with increasing urinary pH. This increased uranium clearance depended in part on an increase in the rate of filtration* and in part on a decrease in the fixation of uranium by the kidney, as shown in Table 5.3.

Table 5.3 — Uranium Clearances by the Rabbit Kidney during Infusion with Various Solutions

Condition of rabbit	Infusion	No. of rabbits	Uranium clearance, % of inulin	Excretion of uranium, μg/min	Filtration of uranium, μg/min	Kidney uranium, μg/g
Oat-fed	Saline	3	28.1	1.55	5.03	65
Normal	Saline	3	66.4	4.27	6.10	25
Normal	0.87% $NaHCO_3$	2	81.0	8.25	8.68	4

The retention of uranium by the kidney quite accurately accounts for the difference between filtration and excretion, the average value of the ratio of the sum of the retention and excretion to the filtration being 105 per cent (Table 5.4).

Table 5.4 — Balance between Filtration, Excretion, and Retention of Uranium by the Rabbit Kidney

Condition of rabbit	Infusion	No. of rabbits	(1) Excretion of uranium, μg/min	(2) Retention of uranium, μg/min	(3) Sum, (1) + (2)	(4) Filtration, μg/min	Quotient, (3) ÷ (4)
Oat-fed	Saline	3	1.55	3.74	5.29	5.03	1.06
Normal	Saline	3	4.27	1.96	6.23	6.10	1.04
Normal	0.87% $NaHCO_3$	2	8.25	0.36	8.68	8.60	1.06

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*Estimated from the inulin clearance, the uranium level in the plasma, and the ultrafilterability of uranium at the existing plasma CO_2 content (see Chap. 1 for a discussion of the latter point).

These studies of the excretion of uranyl uranium by the kidney show that the urinary concentration of uranium is an unreliable indicator of poisoning, the level depending on (1) the amount of uranium taken into the body, (2) the time elapsed since the last exposure to uranium, (3) the excretion of ionic complexers in the urine, and (4) the reaction of the urine. On the other hand, successful demonstration of the presence in the urine of uranium would be evidence of uranium exposure; none of the other tests discussed in this chapter has this specificity.

(c) Uranium and the Kidney. The data in Tables 5.1, 5.2, and 5.3 suggest that uranium in plasma is largely complexed with bicarbonate, in which form it is filterable through the glomerular membrane. As the glomerular filtrate passes down the tubule it is concentrated by reabsorption of water in the proximal convolution, and much bicarbonate of the tubular fluid may be reabsorbed actively.[5] At the same time there would be acidification of the urine by reabsorption of base and a possible simultaneous secretion of acid.[6] Acidification of the urine and reabsorption of bicarbonate would lead to breakdown of much of the bicarbonate-uranium complex, and uranium would be set free to affix itself to the protein of cells lining the distal portion of the proximal tubule. Simultaneous excretion or secretion of organic acids by the kidney would minimize the effect of breakdown of the bicarbonate complex by leading to the formation of complexes between the uranium and the organic anions. Flooding of the tubule with bicarbonate by bicarbonate infusion will prevent breakdown of the complex and deposition of uranium in the kidney (Table 5.3).

Figure 5.1 shows the results of a few determinations of the relationship between the urinary bicarbonate and the plasma bicarbonate levels of the rabbit. The results are similar in a general way to those reported recently for the dog.[7] The scatter of the points is due to the fact that the urinary bicarbonate level is determined not only by the plasma level of bicarbonate but also by the rate of urine flow and by the reaction of the urine, which in turn seems to depend more closely on the alkali reserve than on the amount of plasma bicarbonate actually present. At plasma levels of bicarbonate of less than about 27 millimoles per liter at least 90 per cent of the filtered bicarbonate was reabsorbed in the tubules, whereas at a plasma level of 40 millimoles of bicarbonate per liter only 75 per cent of the bicarbonate that was passed into the glomerular filtrate was reabsorbed by the tubule. The following compilation shows the effect of infusions of various bicarbonate solutions on the reabsorption of bicarbonate by the rabbit's tubules. These data agree very well with some preliminary excretion experiments in which it was found that infusion of 0.44 per cent $NaHCO_3$ into rabbits had no appreciable effect on the excretion and retention of uranyl uranium by the kidney, whereas infusion of 0.87 per cent $NaHCO_3$ markedly increased the excretion of uranium and markedly decreased its retention by the kidney.

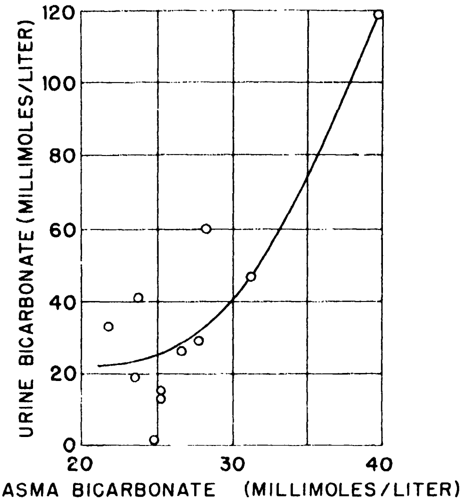

Fig. 5.1—Variation of the urinary level of bicarbonate with that of the plasma in the rabbit.

In a rabbit depleted of base by feeding oats exclusively for several days before the experiment, the percentage of reabsorption of bicarbonate in the tubule was 103 per cent of that of the normal animals.

Infusion of $NaHCO_3$,%	No. of rabbits	Reabsorption of HCO_3 (% of control level)
0.44	2	98.3
0.88	2	85.5
1.0	1	83.8
2.0	1	75.7

Thus the data of Tables 5.1 and 5.3 can be explained now on the basis of changes in the excretion of bicarbonate by the kidney produced by previous feeding on an oat diet or by infusion of bicarbonate. The roughly 15 per cent reduction in bicarbonate reabsorption (leading to increased bicarbonate excretion) produced by infusion of 0.87 per cent $NaHCO_3$ is enough to prevent all but a very slight deposition of uranium in the kidney and to lead to rapid elimination of 80 per cent of the injected uranium from the rabbit's body under the conditions of these experiments. It has not been shown experimentally that bicarbonate infusion will wash uranium out of a kidney in which it has been fixed, but it is known that bicarbonate in vitro will elute uranium from protein. It is possible that bicarbonate would be not only a prophylactic but also a therapeutic agent in poisoning with uranyl uranium.

Since reabsorption of bicarbonate and acidification of tubule fluid take place largely in the proximal convolution, deposition of uranyl uranium would be expected to occur principally in the latter half of the proximal segment of the tubule. Assuming that deposition of uranium is a necessary forerunner of toxic action, the renal functions deranged in uranium poisoning would be expected to be those referable to the proximal convolution of the tubule, and especially those connected with the latter part of this segment.

To check this hypothesis the renal clearances of a number of substances have been examined in the rabbit before and an average of 2 hr and 10 min after poisoning with uranyl acetate. Under these conditions the clearance of glucose, glucose being reabsorbed almost completely in roughly the first half of the proximal convolution,[5] was increased above the control value within 75 min of the injection of uranium, and by 3 hr after the poisoning was 6.5 times the control value. Chloride, urea, and amino acid nitrogen, which are reabsorbed partly by the proximal segment and partly by other portions of the nephron, gave inconstant results: In one rabbit the chloride clearance, for example, increased to 178 per cent of the control value within 2 hr after injection of uranyl acetate, whereas in another animal the chloride clearance 2 hr after poisoning was 91 per cent of the control.

Phenol red and diodrast are substances that enter the urine by both glomerular filtration and tubular secretion, the secretion of diodrast being about twice as great as that of phenol red. The secretion seems to take place in the proximal convolution, and interference with the function of this section of the nephron should result in diminished clearance of these compounds from the blood by the kidney. In fact, it was found that within 2 hr after injection of 2 mg of uranyl acetate per kilogram into a rabbit the diodrast clearance fell to an average of 80 per cent of the control value (Fig. 5.7). The clearance of phenol red behaved in a qualitatively similar manner.

The clearances of inulin, creatinine, and xylose, all of which in the rabbit, as well as in the dog, are excreted entirely by glomerular filtration, would not be expected to be changed acutely by poisoning with uranyl compounds. It was found that in the rabbit there were indeed no significant changes in the clearances of these three substances after injection of uranyl acetate.

Since the proximal convolution is the part of the nephron damaged most extensively by uranyl uranium, a means for evaluating tubular function alone would be very desirable. Calculation of the maximum tubular excretory mass (Tm) is one method by which this could be done, but it is a cumbersome method because it requires titration of the organism. A simpler expression is the transfer of material across the tubular epithelium per unit of substance filtered. For reabsorbed substances, like chloride, a convenient method of calculation is the following (see Sec. 6.1d for a fuller treatment):

$$f_{Cl} = 1 - \frac{U_{Cl}\, P_{In}}{U_{In}\, P_{Cl}} \qquad (1)$$

where U is urinary concentration, P is plasma concentration, and the subscripts Cl and In refer respectively to chloride and inulin. For secreted substances, like diodrast (subscript D) and phenol red, the formula becomes

$$f_D = \frac{U_D\, P_{In}}{U_{In}\, P_D} - 1 \qquad (2)$$

Such an expression, in addition to furnishing a measure of tubular activity uncomplicated by variations in glomerular activity, has the property of being unaffected relatively by inaccuracies of analytical or technical procedure.

Three substances of those mentioned above have been selected for detailed presentation: chloride, diodrast, and inulin, representing, respectively, the classes of materials reabsorbed in the tubule, secreted by the tubule, and unaffected by the tubule. In addition, the excretion of water will be considered briefly.

The typical response of urine flow to intravenous injection of uranyl salts is an immediate increase to an average of 2.1 times the control flow, followed by a slow decrease. With high doses there is a tendency for the urine rate to fall more rapidly from its peak than with moderate doses, as is shown in the upper curve of Fig. 5.2, where the urine rate 2 hr 10 min after poisoning in percentage of the control rate is plotted against the dose of uranyl acetate. For comparison with this curve there is drawn on the same figure a graph derived from the data of Watanabe, Oliver, and Addis[8] on the effect of various doses of uranyl acetate upon the secretion of urine 72 hr after the poisoning. It is of interest that the peaks of both curves come at about the same doses of uranyl acetate, indicating that the doses which have the least immediate effect in reducing urine flow have also the least effect in reducing urine flow 3 days after poisoning. It is noteworthy also that doses causing an acceleration of urine flow 2 hr 10 min after poisoning produce cessation of urine flow in 3 days.

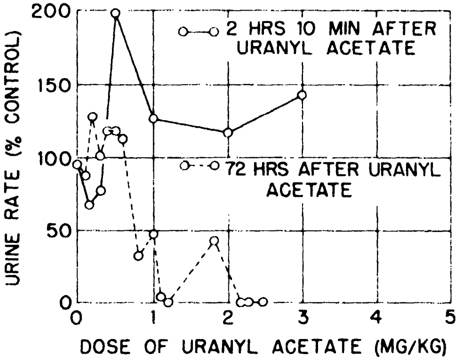

Fig. 5.2—Effect of intravenous dose of uranyl acetate upon the rate of urinary excretion in the rabbit.

In both the rabbit and dog the clearance of chloride in the normal animal is correlated highly with rate of urine flow, the clearance increasing as the urine flow rate increases. A graph of this relationship in the rabbit is shown in Fig. 5.3. In the rabbit both the inulin and diodrast clearances are correlated to some extent with the rate of urine flow, as shown by the lines of Figs. 5.4 and 5.5.

In assessing the effects of administration of uranium compounds upon the clearances of chloride, diodrast, and inulin, the graphs of Figs. 5.3, 5.4, and 5.5 were used to evaluate the expected levels of these variables at the experimental rates of urine flow. The expected values were then compared with the experimentally determined ones. Figures 5.6, 5.7, and 5.8 show the immediate (average, 2 hr 10 min) effects of various doses of uranyl acetate upon these three clearances. It is apparent that only in the case of diodrast clearance is there any progressive effect with increasing dosage. Figure 5.9 shows that there is no immediate progressive diminution in chloride reabsorption although there is in diodrast secretion.

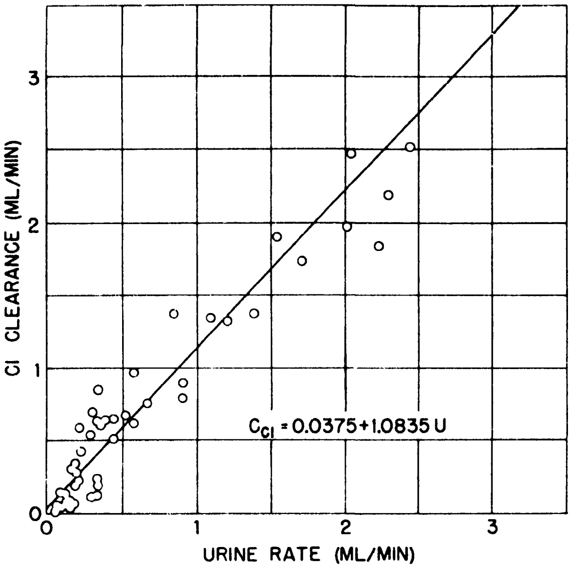

Fig. 5.3—Variation of the renal chloride clearance with the urine rate in the normal rabbit. The line is the one calculated from the indicated data by the method of least squares.

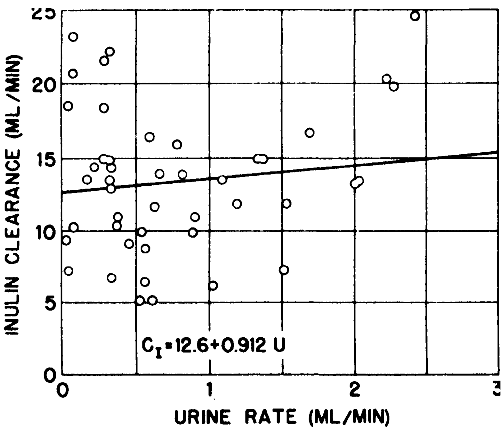

Fig. 5.4—Variation of the renal inulin clearance with the urine rate in the normal rabbit. The least-squares straight line is drawn.

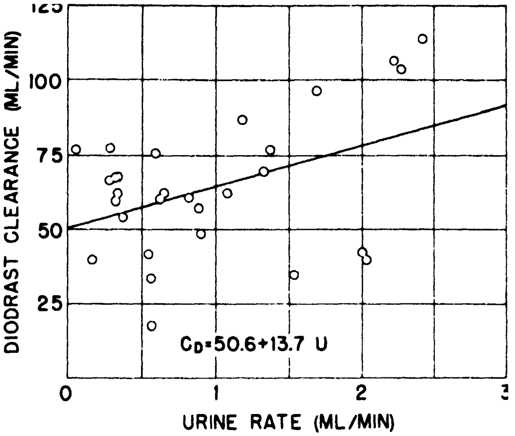

Fig. 5.5 — Variation of the renal diodrast clearance with the urine rate in the normal rabbit. The line is the least-squares straight line.

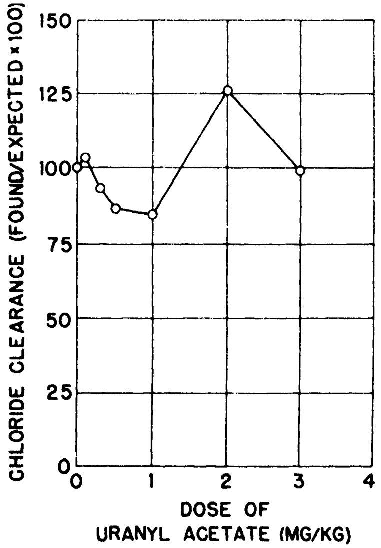

Fig. 5.6 — Immediate effect of intravenous uranyl acetate upon the chloride clearance in the rabbit.

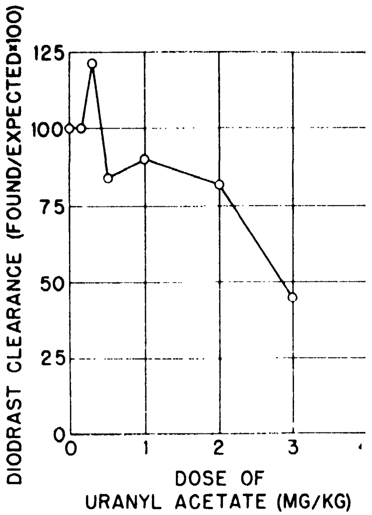

Fig. 5.7—Immediate effect of intravenous uranyl acetate upon the diodrast clearance in the rabbit.

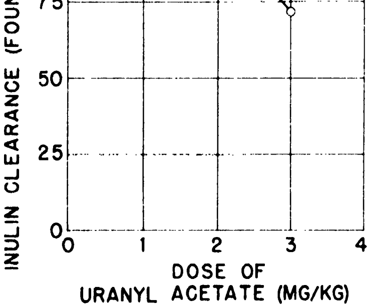

Fig. 5.8—Immediate effect of intravenous uranyl acetate upon the inulin clearance in the rabbit.

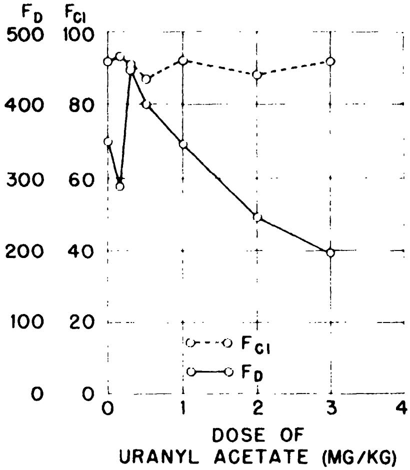

Fig. 5.9—Immediate effect of intravenous uranyl acetate upon the reabsorption of chlorine, F_{Cl}, and the secretion of diodrast, F_D, by the tubule of the rabbit.

The immediate decrease in diodrast secretion could result from decreased blood flow through the kidney, but this is believed not to be the case. It has been observed in the project laboratory by Adler that injection of uranyl acetate does not decrease blood flow through the frog glomerulus. Direct measurements of blood flow through the mammalian kidney by Tribe *et al.*[9] and Bobey *et al.*[10] have shown also that the circulation of the kidney is not decreased after injection of uranyl salts. These facts seem to leave as the only possibility for the reduction of diodrast secretion a direct effect on the diodrast secreting mechanism of the tubule, a mechanism that appears from Fig. 5.9 to differ from that concerned with chloride reabsorption but to be related possibly to that concerned with glucose reabsorption, since both clearances are affected very soon after poisoning.

Figures 5.10, 5.11, and 5.12 show the results of renal-function studies on rabbits exposed to atmospheres of various concentrations of uranyl fluoride dust for different periods of time. It appears from these graphs that the clearance of chloride was not affected particularly by any of the three exposures, although the clearances of diodrast and inulin were reduced definitely by the largest of the dust concentrations used. The large peaks in the uppermost curves of

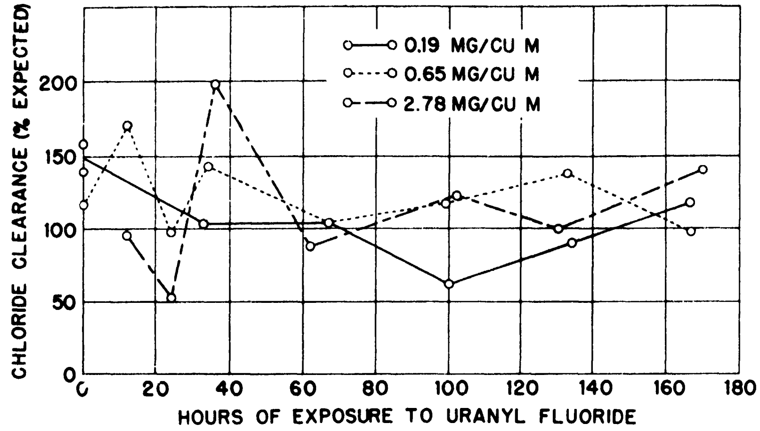

Fig. 5.10—Effects of exposures to atmospheres containing various concentrations of uranyl fluoride dust for different lengths of time (approximately 8 hr/day) upon the chloride clearance of the rabbit.

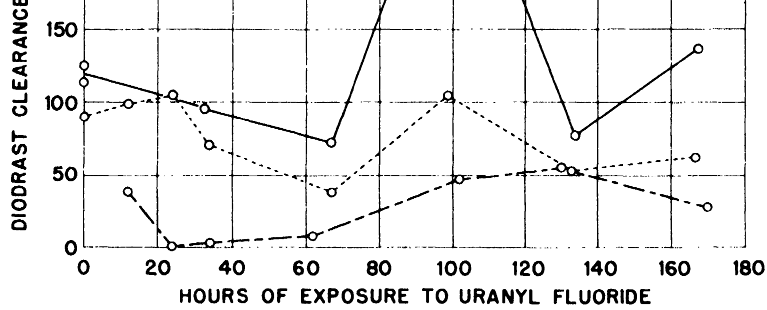

Fig. 5.11—Effects of exposures to atmospheres containing various concentrations of uranyl fluoride dust for different lengths of time (approximately 8 hr/day) upon the diodrast clearance of the rabbit.

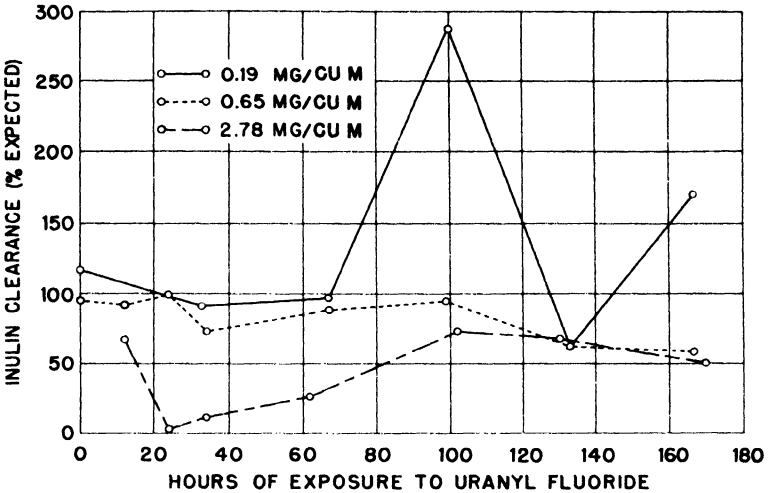

Fig. 5.12—Effects of exposures to atmospheres containing various concentrations of uranyl fluoride dust for different lengths of time (approximately 8 hr/day) upon the inulin clearance of the rabbit.

Figs. 5.11 and 5.12 probably have no special significance inasmuch as each point on these lines represents a different individual. The diodrast and inulin clearances may have been reduced by the intermediate concentration of dust. These effects reached the maximum after 3 days of exposure to the dust (8 hr/day) and then underwent a partial remission, so that there was partial recovery of renal function even during continued exposure to the toxic dust. The fact that clearance of inulin was decreased markedly during the poisoning does not mean necessarily that there was decreased rate of filtration through the glomerulus, for the work of Bobey *et al.*[10] makes it seem possible that, after necrosis of the epithelial cells of the tubule, glomerular filtration may still go on in an approximately normal fashion. The back diffusion of water and dissolved substances from the lumen of the tubule into the peritubular capillaries may be so great, however, that oliguria and possibly anuria ensue. In this manner all renal clearances are lowered toward zero.

When tubular function in these three inhalation experiments is assessed by means of Eqs. 1 and 2, the graphs of Figs. 5.13 and 5.14 are obtained. Figure 5.13 shows that chloride reabsorption was essentially unaffected except by the highest level of dust concentration,

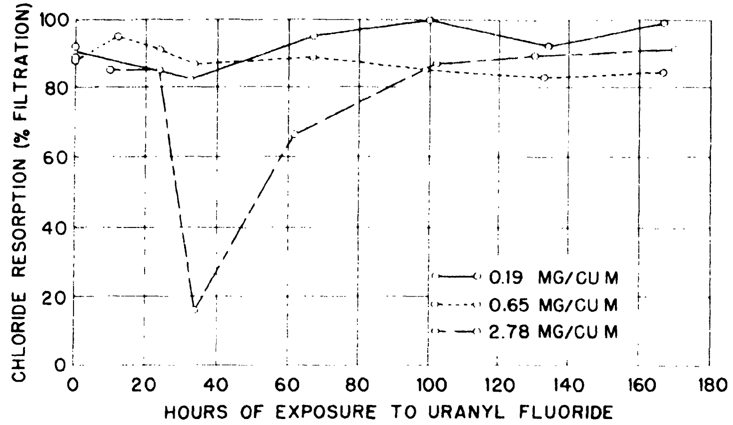

Fig. 5.13—Effects of exposures to atmospheres containing various concentrations of uranyl fluoride dust for different lengths of time (approximately 8 hr/day) upon the reabsorption of chloride by the tubule of the rabbit.

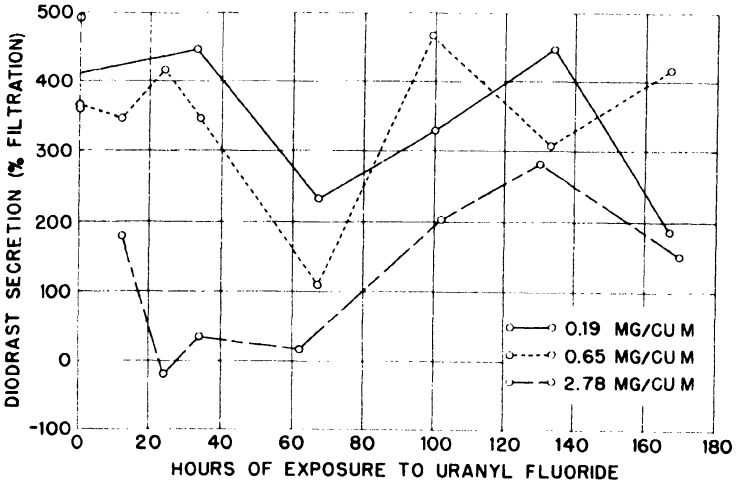

Fig. 5.14—Effects of exposures to atmospheres containing various concentrations of uranyl fluoride dust for different lengths of time (approximately 8 hr/day) upon the secretion of diodrast by the tubule of the rabbit.

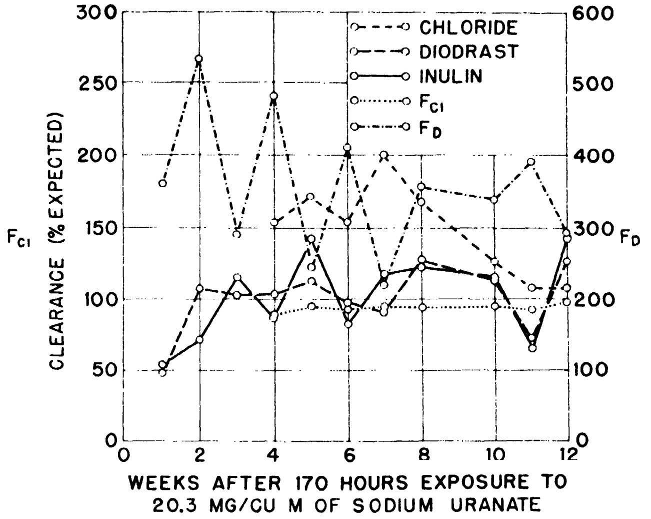

Fig. 5.15—Changes with time of chloride, diodrast, and inulin clearances, chloride reabsorption, and diodrast secretion after cessation of exposure (for about 8 hr/day) to an atmosphere containing 20.3 mg of sodium uranate per cubic meter.

and that the maximum effect appeared after about 4 days (32 hr) of exposure to the dust. In contrast, Fig. 5.14 shows some effect on diodrast secretion by all dust concentrations, the effect being less in magnitude and later in appearance with the smaller dust concentrations. It may be of significance in showing that diodrast secretion and chloride reabsorption occur by different mechanisms in the tubule that the secretion of diodrast reached its lowest point on the third day (24 hr) of exposure, but the low point for chloride came on the fourth day. It is of interest also that chloride reabsorption returned to an essentially normal value during continued exposure to uranyl fluoride dust while diodrast secretion remained low, although it trended toward the normal.

Figure 5.15 shows the results of renal-function studies on a group of animals at various intervals after the cessation of exposure to an atmosphere containing a high concentration of sodium uranate. It can be seen that, although the diodrast and inulin clearances were reduced soon after the end of the exposure, they quickly attained normal levels. Chloride clearance was elevated over the normal value at the earliest time at which it was estimated (4 weeks after the end of

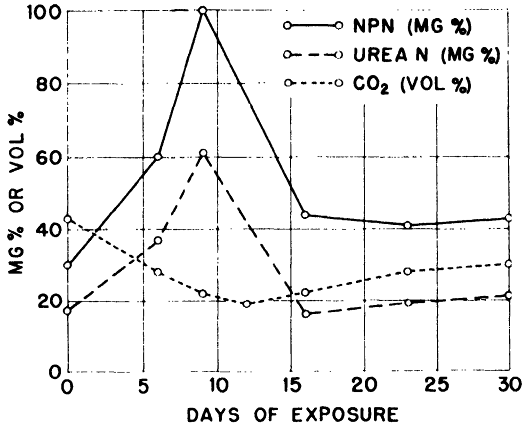

Fig. 5.16—Changes in blood NPN, urea N, and CO_2 content in the rabbit during exposure to an atmosphere containing a mean dust concentration of about 1.0 mg of uranyl nitrate hexahydrate per cubic meter.

exposure), but returned to normal in about 11 weeks from the end of exposure. Secretion of diodrast was essentially normal throughout, so that in this case the early depression of diodrast and inulin clearances may have depended on reduced renal blood flow, since tubular secretion of diodrast and glomerular filtration of inulin were reduced in proportion. Chloride reabsorption was within the normal range wherever studied, so that the elevated chloride clearance probably does not indicate any diminished function on the part of tubular epithelium.

(d) Uranium and the Blood. Failure of reabsorption of chloride by the tubule after administration of uranyl salts (shown in Fig. 5.13) requires a simultaneous failure of reabsorption of base. In severe poisoning the increased urinary excretion of base results in a fall in the amount of base available in the plasma for the formation of bicarbonate. The result is that the CO_2 capacity of the plasma falls to a lowered level, the effect beginning fairly promptly and paralleling in general the altered renal function. In some animals, notably rabbits, the acidosis is accompanied by the appearance of acetone bodies in the urine. A typical graph of the change in plasma CO_2 content during a continuous exposure to inhalation of uranium dust is shown in Fig. 5.16.

The failure of renal function after administration of uranyl salts results in accumulation in the blood of the nitrogenous waste products usually excreted in the urine. The plasma concentration of nonprotein nitrogen may rise to great heights (up to 10 to 15 times the usual value). Usually the increase in urea nitrogen accounts for 65 to 80 per cent of the increase in total nonprotein nitrogen. The percentage of the total nonprotein nitrogen that consists of urea nitrogen increases with the severity of poisoning. The increase in creatinine nitrogen accounts for about 1 per cent. The creatine, uric acid, and amino acid nitrogen fractions make small contributions, but something like 20 per cent of the increase in nonprotein nitrogen is not accounted for by the increases in the plasma concentrations of the nitrogen-containing compounds mentioned. In absolute units this unidentified nitrogen may amount to as much as 70 mg % in plasma. Thus there is a marked increase in the plasma concentration of residual nitrogen. Figure 5.16 contains curves illustrative of the usual courses of the plasma nonprotein nitrogen and urea nitrogen during exposure to uranium by inhalation of a dust-laden atmosphere.

As the plasma concentration of nonprotein nitrogenous substances increases, there is at first little change in the behavior and appearance of the individual. As retention increases, however, muscular weakness and apathy become apparent. Anorexia, possibly associated with vomiting, develops, and there is progressive emaciation. Either constipation or diarrhea may occur in the early stages of uremia; after the onset of anorexia there is failure to produce feces. Finally, coma associated with a fairly marked fall in body temperature sets in and is followed by death. Exitus usually is by respiratory failure.

The relation of azotemia to the uremic syndrome is an unsettled matter. Ingestion by normal subjects of sufficient urea to raise the blood level of urea nitrogen above 70 mg % has been found to produce headache, dizziness, and apathy.[11] Other substances than urea also accumulate in the blood during uremia and may exert some poisonous effect. Guanidine and indican are nitrogen-containing compounds that have been suggested as components of the residual nitrogen fraction of the blood in uremia and as playing a part in the genesis of the uremic syndrome. Phenolic compounds also have been implicated in the production of this symptom complex. It is unlikely that any single factor is concerned in bringing about all the phenomena of the syndrome.[12,13]

The fasting levels of blood glucose in dogs and rats were virtually unaffected by exposure to uranyl nitrate, but the glucose tolerance curve was elevated and prolonged, according to some experiments by E. Roberts.* Table 5.5 gives some of his data for rats exposed for 40 hr to an atmosphere containing 20 mg of uranyl nitrate hexahydrate per cubic meter, each mean representing five rats.

Table 5.5—Effect of Exposure to Uranyl Nitrate Hexahydrate on the Removal of Glucose from the Blood of Rats after Intraperitoneal Injection of 350 Mg of Glucose per Kilogram*

	Blood glucose, mg	
Time	Unexposed	Exposed
Before injection (after 24-hr fast)	65 (53-76)	78 (75-82)
1 hr after injection	133 (112-172)	272 (135-576)
2 hr after injection	105 (98-111)	308 (106-1,055)
3 hr after injection	101 (76-131)	135 (100-184)
4 hr after injection	92 (85-101)	603 (82-1,055)

* Figures in parentheses give the range of values for each mean.

The ability of the animals to convert glucose to liver glycogen was not impaired by the exposure to uranyl nitrate, but there may have been some reduction in the animals' ability to form muscle glycogen. The production of muscle glycogen is comparatively unimportant as a means of removing glucose from the blood, however, and the effect on the formation of muscle glycogen was so slight that it is doubtful that the heightened and prolonged glucose tolerance curve after exposure to uranyl nitrate can be attributed to interference with the polymerization of glucose. Roberts has suggested that the rate of oxidation of glucose by the tissues or the rate of conversion of glucose to fat may be decreased after uranium administration.

Additional light on this subject can be derived from some work of A. Rothstein on the lactate level in blood. The first observation was that during exposure to an atmosphere containing a dust of sodium uranate at a mean level of 19 mg/cu m the blood lactate of the exposed rabbits was consistently about 20 per cent below the control value.

To check this point, an experiment was carried out by Rothstein in which rats were poisoned by intraperitoneal injection of 3 mg of uranyl acetate per kilogram. The animals were divided into groups of five rats each, and the groups were studied before the injection and at intervals thereafter. All animals were fasted for 24 hr before study. Experimental animals received sodium lactate by stomach tube, and control animals received corresponding amounts of saline. Table 5.6 summarizes the results obtained 1 hr after ingestion of the lactate. It is clear from this table that, although the lactate was absorbed into the blood at least partially after poisoning, it was not used in the manufacture of liver glycogen. This finding by Rothstein, coupled with Roberts' report that glucose may not be oxidized so rapidly after poisoning as before, suggests interference with some phosphorylating mechanism, probably not the one involving phosphorylase itself, however, because formation of Cori ester is necessary for glycogen formation from glucose.

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*Experiments referred to in this chapter but not included in the bibliographic references at the end of the chapter were part of work in progress or final reports of the Pharmacology Division of the Manhattan Project of the University of Rochester.

Table 5.6—Effect of Uranyl Acetate on the Utilization of Sodium Lactate Administered to Rats

Days after injection	Increase in liver glycogen due to lactate ingestion, %	Increase in blood lactate due to lactate ingestion, %
0	1,440	196
1	320	89
2	52	362
4	66	
7	13	288

W. F. Neuman has found in rats poisoned by injection of uranyl nitrate that the blood level of inorganic phosphorus was elevated above the control level but that the esterified phosphorus level was relatively unchanged. There was a decreased turnover of radioactive phosphorus by the cephalin, lecithin, and aldehyde lipid fractions of the livers of these animals. All these findings could indicate a slight retardation of tissue phosphorylation.

The serum calcium of rats, which might be expected to fall if the level of inorganic phosphorus rose, showed a slight and probably insignificant tendency to increase in value during exposure to an atmosphere containing 3 mg UF_6/cu m.

In an experiment by Roberts in which four dogs were exposed to an atmosphere containing 20 mg of uranyl nitrate hexahydrate per cubic meter for 51.5 hr in a period of 10 days, the mean serum protein fell slightly from 5.68 g % before exposure to 5.14 g % at the end of the exposure.

R. S. Crossland has found in rats injected intraperitoneally with 5 mg of uranyl nitrate hexahydrate per kilogram that the mean value for the iodine number of the phospholipid fatty acids of the blood increased from 118 before injection to 137 on the fifth day after injection. This difference was significant and may have been correlated with the 15 per cent increase in the iodine number of the phospholipid fatty acids of the liver found also by Crossland. The significance of these changes is unknown; they might indicate enhancement of dehydrogenase activity, inhibition of oxidative mechanisms, or simply transfer of unsaturated acids from the kidney to the liver. Crossland has found that the iodine number of the phospholipid fatty acids of the kidney was decreased by about 25 per cent after poisoning, but this could not explain completely the increases in the blood and liver.

Genaud[14] reported that the blood cholesterol of a dog on a diet of bread was doubled after poisoning with uranyl nitrate, whereas that of another dog on a diet of bread and meat was unaffected. J. Box has found that rats injected with 2.5 mg of uranyl nitrate hexahydrate per kilogram had a lowered level of blood cholesterol on the first day after injection, but on the succeeding days the level was elevated to an average of 1.12 times the control and remained so for about 2 weeks.

The levels of plasma fibrinogen and prothrombin (prothrombin time) have been studied by J. B. Field as indices of toxicity for the liver. Table 5.7 shows some of the data derived from an experiment in which the animals were exposed to an atmosphere containing uranyl nitrate dust. It can be seen from the table that, although the levels of both proteins in the plasma were reduced during exposure, there was a tendency for prothrombin to be affected more than fibrinogen. There was a definite tendency for the fibrinogen levels to return to normal during the exposure but not much evidence of such a trend in the prothrombin levels of the dog. It is probable that the plasma levels of these two proteins are indices of the protein-synthesizing abilities of the liver and therefore can be used to assess interference with normal liver function.

Another, and possibly more specific, test of liver function is the removal of injected bromsulfalein from the blood. A study of this sort has been carried out by Field also. Table 5.8 gives his results on the same dogs represented in Table 5.7, the removals during the first 5 min after injection and the first 30 min after injection being shown. It is apparent that early in the exposure there was decrease in the efficiency of removal of bromsulfalein from the blood by the

Table 5.7—Effect of Inhalation of Uranyl Nitrate Hexahydrate (0.45 Mg U/cu m) on the Plasma Levels of Fibrinogen and Prothrombin

Weeks of exposure	Species	Fibrinogen level, % of control level	Prothrombin level, % of control level
1	Dog	110	97.9
	Rat	86.4	94.9
4	Dog	81.5	95.3
	Rat	80.6	88.8
8	Dog	87.9	84.0
	Rat	103	87.5
12	Dog	92.5	66.6
	Rat	103	87.1
16	Dog	95.7	72.8
	Rat	119	99.6
20	Dog	97.9	66.9
	Rat	112	95.1
24	Dog	90.4	65.6
	Rat	114	106

Table 5.8—Effects of Inhalation of Uranyl Nitrate Hexahydrate (0.45 Mg U/cu m) upon Removal of Bromsulfalein from the Blood of the Dog

Weeks of exposure	Bromsulfalein removal in 5 min, % of control level	Bromsulfalein removal in 30 min, % of control level
1	83	98
4	97	100
8	99	100
12	95	100
16	101	99
20	95	99
24	106	100

liver, but that there was recovery of this function during continued exposure to uranyl nitrate.

Another test in which the removal of a substance from the blood is used as an index of organic integrity is the phenol-red-removal test. It was shown by Eisenbrey[15] that the removal from the blood of injected phenol red varied in the same direction as the urine flow in uranium poisoning. Rothstein has found this test to parallel quite well the tubular secretion of diodrast during exposure to atmospheres containing uranium dusts, the usual course of removal of the dye from plasma under such conditions being illustrated in Fig. 5.17. Since phenol red is excreted chiefly in the urine, the rate of its removal from the blood can be used as an index of renal functional capacity.

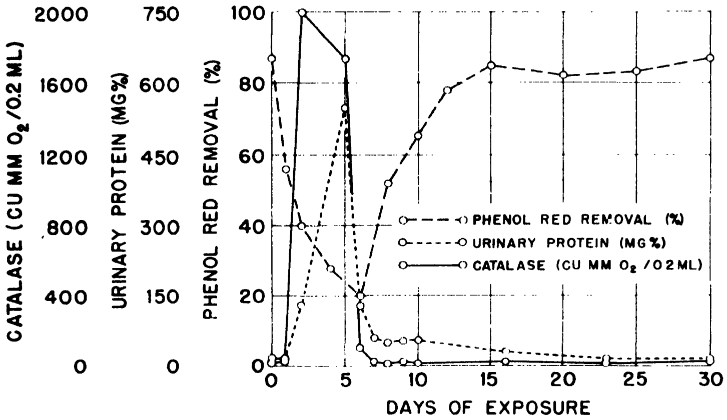

Fig. 5.17—Changes in phenol-red removal from plasma, urinary protein, and urinary catalase in the rabbit during exposure to an atmosphere having a mean dust concentration of about 1.0 mg of uranyl nitrate hexahydrate per cubic meter.

(e) Uranium and the Urine. Urinary excretion of protein begins within a few days after administration of uranyl compounds, the length of time between the dosing and the increased excretion varying inversely with the amount of uranyl salt given. It has been shown that at least some of the urinary protein comes directly from the blood stream (Chap. 10); other protein comes from the breakdown of tubular epithelial cells. The increased urinary level of protein reaches its peak value on about the fifth day of exposure to atmospheric uranium and then returns to a normal value on about the twentieth day of exposure (Fig. 5.17). It is believed that protein can enter the urine by passage through the glomerular capillary wall, by diffusion through the walls of the capillaries of denuded tubules, or by diffusion or expression from the interiors of the injured and desquamated epithelial cells of the tubules. It is thought that in uranium poisoning the protein of the urine gets there largely by the latter two processes. The evidence for this idea will be presented below in connection with the discussion of urinary catalase.

Breedis, Flory, and Furth[16] reported in 1943 that in rats and rabbits uranium poisoning resulted in a greatly increased production of phosphatase in the urine. This observation has been confirmed by A. L. Dounce, who has found that the urinary level of phosphatase is increased in mercuric chloride poisoning also. Dounce has concluded that in the rabbit urinary phosphatase is about as sensitive an indicator of renal toxicity as urinary protein, whereas in the cat and dog urinary phosphatase is much less sensitive than urinary protein.

It appeared to Dounce that, if phosphatase excretion is an adequate indicator of renal toxicity, the urinary level of catalase should be an even better one because there is a comparatively high concentration of catalase in the kidney, and detection of the enzyme is very easy. It was soon found that various nephrotoxic agents led to appearance of large amounts of catalase in the urine, and that the source of the enzyme was probably the kidney itself.

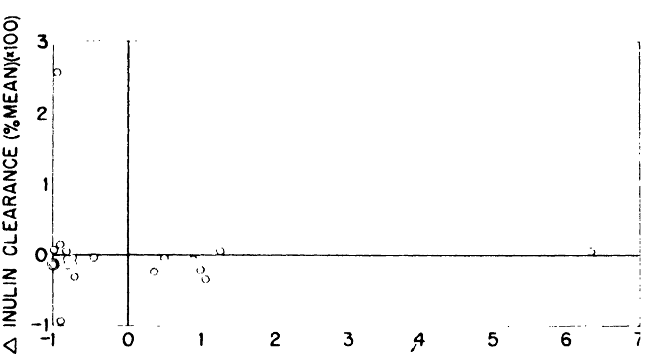

Fig. 5.18—Deviation of catalase clearance from its mean as a function of deviation of inulin clearance from its mean.

The evidence on the latter point is as follows: (1) in normal rabbits there was no correlation between the clearances of catalase and inulin when the former was increased by raising the plasma level by injection of enzyme (Fig. 5.18); (2) in normal rabbits there was no correlation between the plasma and urine levels of catalase; (3) in rabbits poisoned 2 and 4 days previously by intravenous injection of 2 mg of uranyl acetate per kilogram there was no correlation between the plasma and urine levels of catalase after intravenous injection of crystallized catalase; and (4) following fatal poisoning with uranyl acetate the catalase content of rat kidney decreased with time after poisoning. The data about catalasuria will be presented in detail in Chap. 14.

Typical courses of the levels of urinary protein and catalase are shown in Fig. 5.17. It is evident that catalasuria reached its peak more promptly than proteinuria and returned to normal more quickly. Also it will be seen that the relative increase in urinary catalase is greater than that in urinary protein, when the catalase value is expressed as cubic millimeters of oxygen released from a 0.04N hydrogen peroxide-phosphate buffer by 0.2 ml of urine and the protein as milligram per cent.

This relation between the urinary catalase and protein increments has been found to hold for such predominantly tubular poisons as mercuric chloride, mapharsen, and sodium tartrate. It was only when sodium dichromate was used as the kidney poison that the percentage increase in urinary protein level became greater than that in urinary catalase. Since dichromate is known to have its major effect higher in the nephron than uranium and mercury,[17] it is assumed that in dichromate poisoning protein leaks through the glomerulus as well as through and from the injured tubular epithelium. In uranium, mercury, arsenic, and tartrate poisonings, however, the urinary protein comes mainly from the tubule and thus is accompanied by a much greater proportion of catalase, released from injured epithelial cells, than in the case of dichromate.

It has been found that in cats and rabbits urinary-catalase excretion is increased by smaller doses of uranyl salts than is the excretion of protein when the dose of poison is the first one administered; after repeated injections the urinary-protein level may become a more sensitive indicator of renal toxicity than that of catalase. The latter effect may be due to decrease in the kidney-catalase level during the course of repeated injections. In the dog, catalase and protein have just about the same dosage sensitivity.

The excretion of water after administration of uranyl salts shows typically first an increase and then a decrease. The oliguria may proceed to the point of complete anuria. If the kidney has been so severely damaged that it cannot regenerate sufficiently to be able to resume functioning within a reasonable time, the organism dies; if the kidney succeeds in resuming its work, the organism may live. The variations in rate of urine flow after poisoning have been discussed above and illustrated in Fig. 5.2. These changes in the volume output of urine indicate that small doses of uranium or short exposures to larger amounts result in decreased water reabsorption by the tubule, whereas larger doses or longer exposures result in decreased urine excretion. The latter effect could be produced by increased back diffusion of water and dissolved materials from the tubule lumen into the blood stream through the denuded tubule wall.

As the daily urine volume increases in the early stages of poisoning there is an inverse change in specific gravity. When the urine volume decreases again as the severity of the renal effect increases, there is no increase in the specific gravity, however. With recovery from the poisoning the kidney recovers its concentrating ability.

The pH of the urine increases immediately after poisoning but decreases terminally, as shown in Table 5.9, based on work by F. L. Haven. The initial rise in pH probably reflects the initial increased excretion of base that results in lowering of the CO_2 content of plasma. The final acidity of the urine may indicate either depletion of the alkali reserve of the body, excretion of organic acids, or both.

Table 5.9—Effect of Injection of Uranyl Nitrate Hexahydrate (5 Mg/kg) upon Water Excretion and Urine pH in the White Rat

Days after injection	No. of rats	Urine production, % of control	Urine pH, % of control	Urine pH
1	20	193	110	6.7
2	20	249	106	6.5
3	20	293	92	5.6

C. Randall has shown that following injection of uranyl nitrate into rats there is a much increased urinary excretion of citrate, and Rothstein has found that rabbits exposed to inhalation of sodium uranate showed a tripling of the urinary output of lactate. H. E. Stokinger and H. P. Dygert have reported that rabbits exposed to inhalation of uranium dioxide increased their mean daily urinary excretion of β-hydroxybutyrate from 1.18 to 2.37 mg on the fourth day of exposure and increased their mean daily excretion of total acetone bodies from 1.19 to 2.58 mg. The daily ascorbate excretion of rats increased to 6.6 times the basal value after poisoning. Thus there is considerable evidence for an increased urinary excretion of organic acids. It has been shown already that there is a decreased alkali reserve, so that both factors mentioned above appear to contribute to the lowering of urinary pH after poisoning.

The increased excretion of ketone bodies suggests some impairment of the oxidative mechanisms of the liver. Another suggestion of interference with liver function comes from studies of phenol excretion by the rat, carried out by J. F. O'Leary. Some data from this experiment are shown in Table 5.10, from which it can be seen that in the first days after poisoning the urinary excretion of total phenols generally increased, whereas that of conjugated phenols generally decreased. Later both conjugated and total phenols tended toward the control values. The decreased ratio of conjugated to free phenol after poisoning may indicate interference with liver function, since the conjugation of phenol is thought to occur in that organ. On the other hand, impaired liver function cannot be the only factor involved, because the total excretion of phenols is increased. It may be that the increased phenol excretion is a reflection of tissue destruction following the poisoning, involving the production of hydroxy acids like phenylacetic or *p*-hydroxyphenylpropionic, which do not require conjugation.

Table 5.10—Effect of Injection of Uranyl Nitrate Hexahydrate upon the Urinary Excretion of Phenol by the Rat

Dose, mg/kg	Phenol excretion after 4 days, % of control		Phenol excretion after 8 days, % of control	
	Conjugated	Total	Conjugated	Total
0.32	82	124	109	107
0.63	75	112	99	87
1.25	97	133	96	92
2.50	115	145	116	112

The excretion of nonprotein nitrogen fractions in the urine has been studied fairly extensively because of its bearing on the genesis of nitrogen retention. In one experiment carried out by Rothstein, rabbits were injected with 0.75 mg of uranyl acetate per kilogram, and the composition of the urine and blood followed thereafter for a period of 10 days. The results are shown in Table 5.11. It is evident at once from this table that, although there was a tendency for the urinary concentrations of most fractions of the nonprotein nitrogen to decrease if they underwent any change, there was a large increase in the urine concentration of amino acid nitrogen.

The explanation for this difference may lie in the fact that amino acid nitrogen seems to be reabsorbed actively in the proximal tubule, like chlorides, whereas other fractions of the nonprotein nitrogen are reabsorbed passively. Thus increased concentration of amino acid nitrogen could occur after interference with the function of the proximal tubule, whereas decreased concentration of the other nonprotein nitrogen fractions would follow injury of the tubular epithelium and the consequent increase in the permeability of the tubule to passive diffusion of substances from the lumen to the peritubular capillaries.

In other experiments in which actual excretion was studied as well as urinary concentration, Rothstein concluded that the decreased excretion of nonprotein nitrogen substances was sufficient to account for the azotemia found experimentally. It is noteworthy in Table 5.11 that, although the urine concentrations of total nonprotein nitrogen, of urea nitrogen, and of creatinine nitrogen were depressed on the second day after injection, the blood level of nonprotein nitrogen did not rise appreciably until after the second day of the experiment. This shows clearly that the rise of blood nonprotein nitrogen is not a primary effect but is secondary to decreased urinary excretion.

Table 5.11 — Effect of Injection of 0.75 Mg of Uranyl Acetate per Kilogram upon Retention of Nonprotein Nitrogen by the Rabbit

(In percentages of control levels)

	Days after injection							
	1	2	5	6	7	8	9	10
Blood NPN		111	294			305		
Urine NPN	110	60	43	64	111	163	206	112
Urine urea N	127	45	46	68	50	85	100	116
Urine creatinine N	144	72	65	68	84	98	136	136
Urine amino acid N	129	383	362	122	197	210	134	197

The excretion of ammonia by the kidney is considered to parallel in general the ability to excrete urea.[18] However, in one of Rothstein's experiments in which rabbits were exposed to an atmosphere containing 20 mg of sodium uranate per cubic meter, the excretion of ammonia increased whereas that of urea decreased, as seems to have been found first in uranium poisoning by Přybil[19] and Pipat[20] in 1929. On the other hand, a group of rats injected with uranyl acetate by Rothstein showed no increase in urinary excretion of ammonia nitrogen, and a group of rabbits exposed to an atmosphere containing 18 mg of uranium tetrachloride per cubic meter (21 per cent of which changed to uranyl chloride in the chamber according to analysis) showed less increase in ammonia excretion than rabbits placed in the exposure chamber but unexposed to uranium-bearing dust.

The variable effect of uranium on urinary excretion of ammonia might mirror a variable action on the production of acids in the body, the urinary excretion of ammonia being possibly a response to acid excretion.[21] Gamble, Ross, and Tisdall[22] have suggested that the stimulus to ammonia excretion is a lowered alkali reserve rather than an increased hydrogen-ion concentration in the blood. Both of these possible stimuli to ammonia excretion seem to be present during uranium poisoning, so that the best guess about the cause of the variable effect on ammonia excretion is that in toxic states there is a balance between those changes in the animal which stimulate ammonia excretion (increased acid production or decreased alkali reserve) and those which prevent its appearance (parenchymal damage).

The large increase in the urinary concentration of amino acid nitrogen furnishes another method for testing renal function. This can be

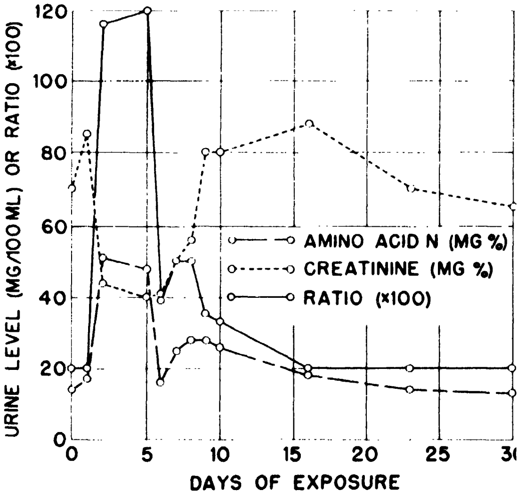

Fig. 5.19—Changes in the urinary concentrations of amino acid nitrogen and creatinine and in the value of the ratio of the former to the latter in the rabbit during exposure to an atmosphere having a mean dust concentration of about 1.0 mg of uranyl nitrate hexahydrate per cubic meter.

set up as follows: In the normal kidney the renal clearance of creatinine can be used as a measure of glomerular filtration, and the urine level of creatinine can serve as a rough index of the extent to which the glomerular filtrate has been concentrated in the tubule.

Then the ratio of the concentration of any constituent of normal urine to that of creatinine gives a figure that should be relatively independent of urine flow. If the substance is one that normally is reabsorbed by the tubule cells, like amino acid nitrogen, any increase above the normal figure for the ratio will mean decreased reabsorption; a decrease in the value of the ratio will indicate increased reabsorptive activity by the tubule cells. The ratio of the concentrations in the urine of amino acid nitrogen and creatinine has been found to be more reliable as an indicator of altered renal function than either urine level alone (Fig. 5.19).

(f) Uranium and the Liver. Certain evidences of some effects of uranium on the liver have been mentioned already: the decreased plasma levels of fibrinogen and prothrombin, the decreased removal of bromsulfalein from the plasma, the increased excretion of ketone bodies, possibly the increased excretion of free phenol, and possibly the failure of conversion of injected lactate into liver glycogen. In addition to these findings, there is a report by Haven that rats killed 21 days after injection of 2.5 mg of uranyl fluoride per kilogram had total liver lipid contents of 16.9 per cent of the dry weight compared with 14.2 per cent for control animals. This confirms Hunter's impression that the livers of his rabbits were fatty.[23] It is probable that such effects are secondary to the renal action because they seem not to precede altered renal function. MacNider[24] found that histological alteration of the liver paralleled the acidosis rather than the dose of uranyl salt given, showing the secondary character of the effects observed by him in the liver.

3. CONTROL VALUES

Table 5.12 presents extreme values in control animals for the various parameters discussed here.

Table 5.12 — Ranges of Various Parameters in Control Animals

	Dog		Rabbit		Rat	
	Min.	Max.	Min.	Max.	Min.	Max.
Blood NPN, mg%	22	50	22	55		
Blood urea N, mg%	6	18	7	25		
Blood creatinine, mg%	0.9	2.1	1.2	2.3		
Blood uric acid, mg%	0.3	0.5	0.2	1.0		
Blood amino acid N, mg%			8.9	12.6		
Blood glucose, mg%	70	90	50	80		
Blood lactic acid, mg%			16	40		
Blood calcium, mg%	10.9	12.9	14.9	17.5		
Blood phosphorus, mg%	3.6	5.3	4.9	7.8		

Table 5.12 — (Continued)

	Dog		Rabbit		Rat	
	Min.	Max.	Min.	Max.	Min.	Max.
Plasma CO_2 content, vol.%	34	54	35	63		
Iodine No. plasma fatty acids						117[§]
Plasma protein, %	2.8	8.7				
Plasma fibrinogen, mg%	275	450	250	425	250	425
Prothrombin time, sec	20	27	19	28	36	42
Bromsulfalein removal, %	85	100	85	100		
Phenol red removal, %	85	100	85	100		
Urine NPN, mg%			200	2,340		
Urine urea N, mg%			120	1,465		
Urine amino acid N, mg%			5	35		
Urine creatinine, mg%			30	150		
Amino acid N/creatinine	0.8	1.9	0.1	0.25		
Urine creatine, mg%			1	20		
Urine ammonia, mg%			0	2		
Urine protein, mg%	0	10	0	30		
Urine catalase, $mm^3 O_2/0.2$ ml	0	50	0	50		
Urine phosphatase, units%	0	5	0	3		
Urine glucose, mg%			300	700		
Urine lactic acid, mg%			8	25		
Ketone body excretion, mg/day			0.6	1.3		
Phenol excretion, mg/day					9.4	16.5
Vitamin C excretion, mg/day					0.1	0.4
Daily urine vol., ml			75	200	5.1	10.9
Urine specific gravity	1.006	1.062				
Urine pH	5.20	7.30	5.60	8.00	6.0	6.4
Chloride clearance, ml/min	0.1	4.0	0.05	2.51[*]		
Diodrast clearance, ml/min	160	320	32.0	115[†]		
Inulin clearance, ml/min	43	90	5.0	24.5[‡]		

[*] See Fig. 5.3.
[†] See Fig. 5.5.
[‡] See Fig. 5.4.
[§] Mean figure.

4. SUMMARY

Evidences of interference with the normal functions of both the liver and kidney in poisoning with uranyl salts have been found. In general the evidences of liver dysfunction have not been particularly marked, whereas those of kidney dysfunction have been quite striking and in general have preceded in time the appearance of liver effect. It appears possible that the kidney is the only parenchymal organ affected directly by uranium, and that the effects on other organs are consequent to the acidosis and azotemia, with attendant cachexia, induced by the kidney dysfunction. For this reason tests of kidney integrity seem the most pertinent to uranium poisoning as diagnostic aids.

Of the possible tests of renal integrity, the urinary excretions of catalase, protein, and phosphatase have the same significance; they indicate an active process of cellular breakdown within the kidney. Proteinuria may indicate also breakdown of some of the normal barriers between blood plasma and urine. In general, these tests show a sharp peak 2 to 5 days after the acute insult and thereafter a quick return to normal levels. Catalase appears dependably in the urine of the rabbit after single intravenous injections of 0.02 mg of uranyl acetate per kilogram.

The tests of functional capacity of the kidney tubule (phenol-red removal from the blood, the ratio of the urinary excretions of amino acid nitrogen and creatinine, chloride and diodrast clearances, chloride reabsorption, and diodrast secretion) show their maximal change 3 to 6 days after the acute insult by uranyl uranium. Even during continued exposure to uranium the kidney function returns toward normal if the exposure is not sufficiently great to produce rapid fatality. After heavy exposures the chloride clearance and the amino acid nitrogen-creatinine ratio may remain elevated for appreciable periods of time after cessation of the exposure, indicating some prolonged interference with tubular activity. The ratio of the urinary concentrations of amino acid nitrogen and creatine has been found to be elevated consistently in rabbits by injection of 0.05 mg of uranyl acetate per kilogram and sometimes by injection of 0.03 mg/kg. The other tests of renal function have not been studied in this way, but their dosage sensitivity would be expected to be about the same as that of the ratio.

PART B. METHODS

In this section it is proposed to give satisfactory methods for carrying out the analyses and techniques mentioned in Part A. First mention will be given to those procedures of use in testing renal function and integrity directly; then methods for following the direct consequences of renal dysfunction will be described; finally methods for studying the secondary effects of renal dysfunction will be given.

5. RENAL INTEGRITY

5.1 Alkaline Phosphatase Concentration of Urine. In a test tube 0.5 ml of urine and 5.0 ml of veronal buffer (11.5 g of sodium veronal in 500 ml of water) are mixed and allowed to come to temperature equilibrium at 37°C. Then 0.5 ml of a well-mixed 4 per cent suspension of calcium phenolphthalein phosphate is added. The tubes are shaken well and allowed to stand at 37°C for 1 hr. Then the supernatant solution is poured off.

To the separated supernatant fluid 0.1 ml of 1.5N NaOH is added; the mixture is centrifuged if cloudy. The color of the solution is read in a Klett colorimeter with a filter having maximum transmission at 540 mμ, the zero being set with a blank (centrifuged if necessary) containing 0.5 ml of urine, 5.0 ml of veronal buffer, 0.5 ml of water, and 0.1 ml of NaOH. The colorimeter readings are converted to King-Armstrong units per 100 ml of urine by means of a calibration curve (1 scale division = 0.043 King-Armstrong unit per 100 ml of urine).

5.2 Protein Concentration of Urine. The protein content of the urine can be estimated conveniently by the sulphosalicylic acid method of Kingsbury, Clark, Williams, and Post[25] as described by Hall and Gault.[26]

5.3 Catalase Concentration of Urine. Three more or less satisfactory methods are now available for the determination of catalase in either plasma or urine. These are the titrimetric method,[27] the manometric method with the Warburg apparatus, and the manometric method with the apparatus devised by Dounce. The two manometric methods have about the same limits of sensitivity, but Dounce's apparatus is much more compact and portable than that of Warburg. The manometric methods will detect about one-tenth as much catalase as the titration method and have been used exclusively in the work described here.

The Warburg apparatus is used in the usual manner for measuring gas production.[28] Two milliliters of the peroxide-phosphate buffer described below is placed in the flask, 0.05 ml of 3N KOH in the central well, and 0.2 ml of the fluid under examination in the side arm. The flask is equilibrated at 25°C and shaken for 15 min after mixing.

The essential parts of the Dounce apparatus are the manometer assembly (Fig. 5.20) and some device for oscillating the manometer assembly about a horizonal axis at its upper end at a rate of about 250 oscillations per minute.*

The bulb of the apparatus is cleaned after each use with 1:5 NH_4OH followed by thorough rinsing with distilled water. The manometer also is flushed out with distilled water. Every week or two the manometers and bulbs should be cleaned with hot chromin acid cleaning solution and then rinsed exhaustively. The glass beads used in the apparatus are cleaned in the same way as the bulbs.

A clean glass bead is placed in the bottom of the bulb, and 0.2 ml of urine or other solution is measured into the bulb. Then enough

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*A satisfactory shaking apparatus is described in the appendix to this chapter.

0.04N H_2O_2 in 0.0066M phosphate buffer at pH 6.9 is added to the bulb to raise the liquid level 1 to 2 mm above the bottom of the ground joint.

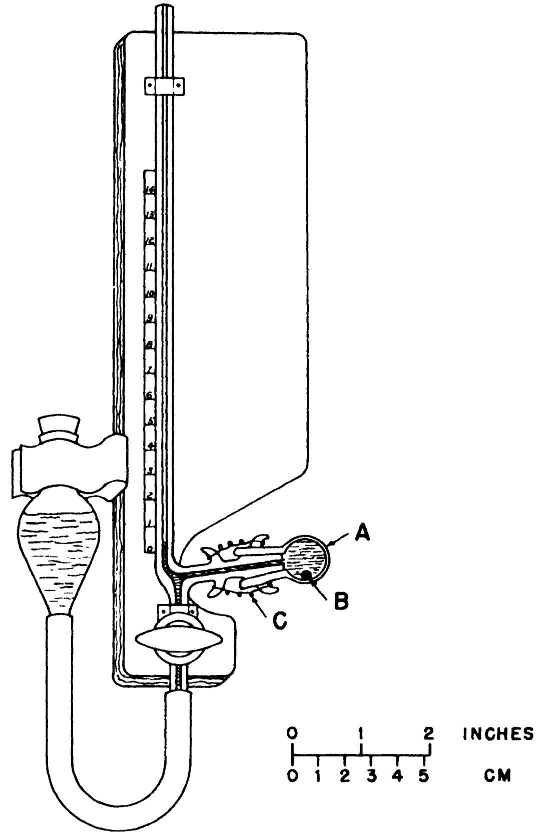

Fig. 5.20—Manometer assembly of the Dounce apparatus. The manometer, made of capillary tubing with a 1-mm bore, is mounted on a wooden block ¾ in. thick. The bulb A contains just 1.5 ml of liquid when filled to the neck without the glass bead B. The bulb is fastened to the manometer by springs C.

The manometer block, held in the left hand, is turned on edge with the ground joint down, and the capillary filled with water by opening the stopcock. When water runs from the end of the ground joint, one finger of the left hand is placed over the top of the manometer to block the lumen, and the stopcock is closed. The bulb is then raised carefully up over the male portion of the ground joint until the bottom of the joint barely clears the surface of the liquid in the neck of the bulb. Then the joint is seated by a sudden jamming together of the two parts. It is important that no air bubbles be introduced into the capillary during this procedure.

The bulb is fastened to the manometer with springs or circles of rubber tubing, and a stop watch is started. The level of liquid in the manometer is brought to zero by use of the leveling bulb, and shaking of the apparatus is started. After 15 min by the stop watch the shaking apparatus is stopped, and the manometer is read. The reading in millimeters multiplied by 1.13 gives the cubic millimeters of oxygen evolved in 15 min, provided that the machine is operated at a temperature near 25°C and that the capillary has a bore of 1 mm.

The analysis should be carried out as soon after collection of the urine sample as possible (within 25 min), and the samples should be free of hemoglobin or red cells. Urine samples can be stabilized by the addition of 5 ml of a 20 per cent solution of gum arabic (brought to pH 5.5 to 6.0 with 1M NaOH) to 95 ml of urine. When so prepared, urine can be stored at room temperature for 6 to 8 hr without serious loss of activity.

A standard catalase solution should be available for checking both the Warburg and the Dounce apparatus from time to time. Crystallized beef-liver catalase can be prepared by the method of Sumner and Dounce.[29] A solution having a k value of 0.008 per ml (standardized by the method of Euler and Josephson[30]) should give a reading on the Dounce machine of about 50 mm. It is convenient to prepare a more concentrated stock solution that can be stored in a refrigerator and from which the working standard can be prepared by dilution.

6. RENAL FUNCTION

6.1 Clearance Determinations. (a) Rabbit. Clearance determinations have been made on rabbits only in sacrifice experiments. The animal is anesthetized with veterinary nembutal solution, the dose being determined from a curve similar to that of Bazett and Erb[31] but shifted downward so that a rabbit of 3 kg body weight receives 1.8 ml of nembutal solution. Additional anesthetic is given as required to maintain an approximate plateau of anesthesia (in a 3-kg rabbit, injection of 0.2 ml of the solution every 30 min usually has the desired effect).

Appropriate cannulas are tied into the trachea, the left saphenous vein, and the bladder. As soon as the venous cannula has been put in place, infusion of the animal with an appropriate solution containing inulin (0.25 per cent) and diodrast (0.52 vol. % of 30 per cent solution) is started at the rate of 25 ml/kg/30 min. After priming for 30 min, the infusion rate is lowered to 1 ml/min for the duration of the experiment. Meanwhile the bladder cannula has been inserted, and the deep femoral artery of the right leg has been dissected out.

After an equilibration period of at least 1 hr following the end of priming, collection of successive urine samples at 30-min intervals is started. Arterial blood samples are drawn from the deep femoral artery at the mid-points of the urine collections. At least two determinations are made on each animal.

(b) Dog. Repeated determinations have been made on the same animal, no dissection or anesthesia being required. The animal (female) lies on one side on a table, restraint ties being used if necessary. The dog is catheterized with a wing-tip soft rubber catheter, and a needle is taped in place in one leg vein.

The dog is primed by infusion for 30 min at the rate of 3 ml/min with a sterile solution containing inulin (0.7 per cent) and diodrast (1.4 vol. % of 35 per cent solution). At the end of the priming period, infusion is continued at the same rate but with a solution containing half the preceding concentrations of inulin and diodrast. Equilibration is allowed for at least 1 hr after the end of the priming period before collection of clearance samples is started. Four consecutive urine samples are collected at 15-min intervals, the bladder being washed out with 10 ml of warm distilled water at the end of each period. A venous blood sample is taken at the mid-point of each collection period.

(c) Analytical Methods. Urine and plasma samples are analyzed for chloride by methods given on pages 833 and 834 of Peters and Van Slyke[32] or by the procedure of Schales and Schales.[33] Their inulin and diodrast concentrations are estimated by the methods of Harrison[34] and Alpert.[35]

(d) Calculations. The various urinary clearances C are calculated from the analytical data and the urine volume by the following formula:

$$C = \frac{uU}{Pt} \tag{3}$$

where u is the volume of urine (plus washings in the case of the dog) collected in t min. U is the concentration of substance in the urine, and P that in plasma.

For estimation of tubular function, the following scheme has been set up. In the case of a substance secreted by the tubule, the amount of blood cleared by secretion is the difference between the clearance of the substance and that of inulin. When the secretory activity is related to the filtration, we have in the case of diodrast:

$$\frac{C_D - C_{In}}{C_{In}} = \frac{C_D}{C_{In}} - 1 \qquad (4)$$

This reduces to $\frac{U_D\ P_{In}}{U_{In}\ P_D} - 1$, which is Eq. 2, when the determined values are inserted. For reabsorbed substances, like chloride, the expression in Eq. 1 is derived in the same way.

6.2 Urinary Amino Acid Nitrogen-Creatinine Ratio. The urinary level of amino acid nitrogen is estimated by the copper method[36] and that of creatinine by Folin's method.[37]

6.3 Phenol-red Removal. As carried out in rabbits, this test consists in intravenous injection of 3 mg of phenol red as the sodium salt. Venous blood samples are drawn 3 and 30 min after the injection, and the oxalated plasmas examined for their dye content by alkalinization and colorimetric comparison with suitable standards. The percentage of removal of the dye is calculated by multiplying by 100 the ratio of the difference between the two plasma levels to the level of 3 min.

6.4 Urinary Excretion of Various Substances. (a) Nitrogenous Substances. Urinary NPN can be determined after precipitation of proteins with either trichloracetic acid or tungstic acid[38] by the method of Wong.[39] Urine urea N can be determined by the aeration method of Van Slyke and Cullen[40] or by microdiffusion.[41] Creatine in urine can be determined by the open-flask method of Folin[37] or by the more direct method of Eggleton *et al.*[42] Urine ammonia is readily determined by microdiffusion.[41]

(b) Carbohydrate Substances. Urinary glucose can be estimated by a variety of methods (see bibliographic references 43 to 46). Lactic acid in urine can be determined by the method of Barker and Summerson[47] or by Koeneman's modification[48] of the Miller-Muntz method. Pyruvic acid can be estimated by the procedure of Friedemann and Haugen.[49] Citric acid in urine is determined according to Pucher *et al.*[50]

Total acetone bodies in urine can be determined by the method of Crandall[51] after removal of interfering substances by precipitation with copper sulfate and calcium hydroxide. Acetone and diacetic acid can be determined by the same procedure except that potassium dichromate is not added and the period of refluxing is reduced to 40 min.[52] Beta-hydroxybutyric acid is determined by difference.

Urinary phenols can be estimated by the colorimetric molybdate-tungstate method of Folin and Denis,[53] and ascorbic acid in urine can be determined by either the 2,6-dichlorophenolindophenol method,[54] the 2,4-dinitrophenylhydrazine method,[55] or the phosphotungstic acid method.[56]

7. CHANGES INCIDENT TO DECREASED RENAL FUNCTION

7.1 Nitrogen Retention. Total nonprotein nitrogen of blood can be estimated by the Folin-Wu method,[57] by the semimicro modification of Irving and Forbes,[58] by the method of Koch and McMeekin,[59] or by microdiffusion.[41] Blood urea can be determined by a number of methods; convenient ones are those of Folin and Svedberg,[60] Folin and Sumner,[61] Van Slyke and Cullen,[40] and Conway.[41] Blood creatinine and creatine can be determined by Folin's method,[62] and blood uric acid by the methods of Benedict and Behre,[63] of Folin,[64] and of Blauch and Koch.[65] The blood level of amino acid nitrogen can be estimated by the procedure of Frame *et al.*[66] as modified by Russell.[67]

7.2 Other Blood Constituents. Blood glucose can be determined by many methods; some of the better ones are those of Folin and Wu,[68] Folin and Malmros,[69] Van Slyke and Hawkins,[44] Shaffer and Hartmann as modified by Somogyi,[70] Benedict,[71] Nelson,[46] Sumner and Sisler,[72] and Schales and Schales.[73] Blood lactic acid can be estimated by either of the methods mentioned previously.[47, 48]

Calcium in serum or plasma can be determined conveniently by the methods of Roe and Kahn,[74] or of Rappaport and Rappaport.[75] The method of Fiske and Subbarow[76] can be used for determination of phosphorus in whole blood, plasma, or serum. Other easy procedures for estimation of blood phosphorus are those of Benedict and Theis[77] and Sumner.[78] Plasma CO_2 content can be estimated either by the gasometric methods of Van Slyke[32] or by the microdiffusion method.[41] Blood phenols can be determined by at least three methods.[79,80,81]

8. CHANGES CONSEQUENT TO DECREASED RENAL FUNCTION

8.1 Plasma Proteins. Total plasma protein can be determined colorimetrically,[82] turbidimetrically,[83] or by Kjeldahl analysis.[84] Plasma fibrinogen is estimated by the method described by Reiner.[85] Prothrombin clotting time is determined by the method of Campbell *et al.*[86] using both undiluted and 12.5 per cent plasma. The values for diluted plasma appear to be the most satisfactory.

8.2 Plasma Lipids. Cholesterol in blood can be determined by such methods as those of Bloor, Pelkan, and Allen;[87] Kelsey;[88] or Forbes and Irving.[89] The phospholipids of the plasma can be determined by the method of Bloor.[90] The iodine number of the fatty acids can be measured by the procedure of Yasuda.[91]

8.3 Bromsulfalein Removal from the Blood. This test consists of intravenous injection of 2 mg of bromsulfalein per kilogram and withdrawal of blood samples at 5 and 30 min after the injection. The amount of dye remaining in the plasma at these two times is estimated colorimetrically by the method used by Rosenthal and White.[92] In normal animals 15 per cent of the dye remains 5 min after injection, and none is present after 30 min. Lower values for bromsulfalein removal indicate interference with the excretory activity of the liver.

9. APPENDIX

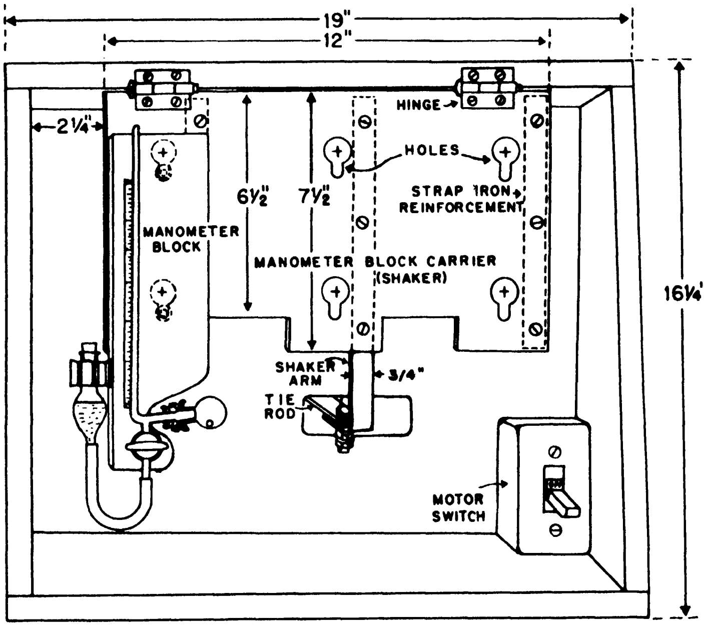

Fig. 5.21—Front elevation of shaking apparatus, showing one manometer assembly in position.

Shaker for the Dounce Catalase Apparatus. The construction of a shaking apparatus for use with Dounce catalase manometers is illustrated in Figs. 5.21 to 5.23. As shown in Fig. 5.21, this particular machine carries three manometer assemblies. These are attached to the manometer block carrier by a key-and-slot arrangement, shown in detail in Fig. 5.22. The motor shown in Figs. 5.22

and 5.23 is a variable-speed governor-controlled electric motor (specification No. R35E of the Lee Engineering Co., Milwaukee, Wis.). A motor with a fixed speed of about 250 rpm and an output of $^1/_{10}$ to $^1/_8$ hp would be satisfactory. The connections from the junction box to the motor (Fig. 5.23) are run through a short piece of metal conduit to prevent abrasion of the wires by moving parts. The entire apparatus, including three manometer assemblies, can be fitted into a case that can be transported, like a suitcase, by a single person. Thus the apparatus is readily portable.

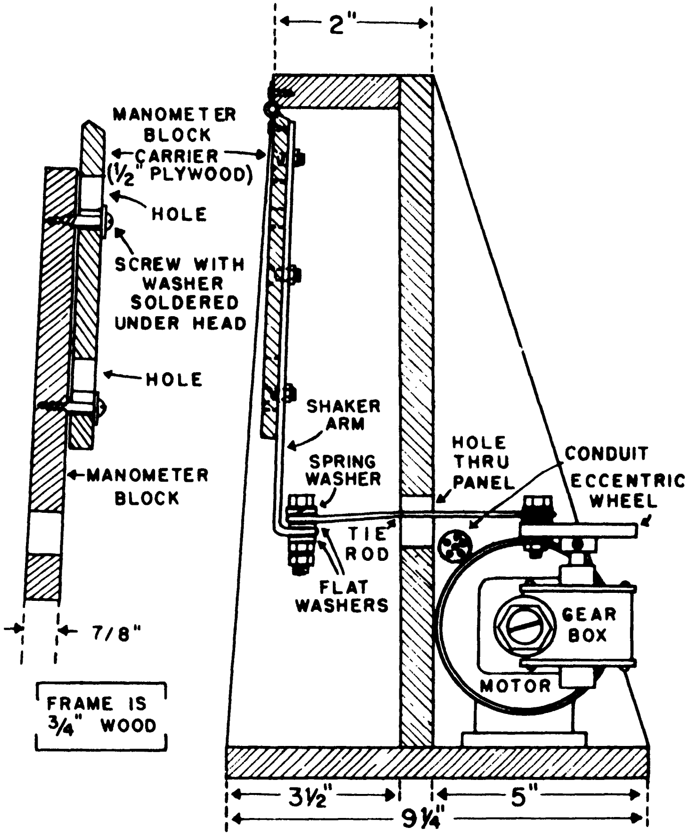

Fig. 5.22—Vertical section of shaking apparatus and detail (left) of key-and-slot arrangement for mounting the manometer assembly on the manometer block carrier.

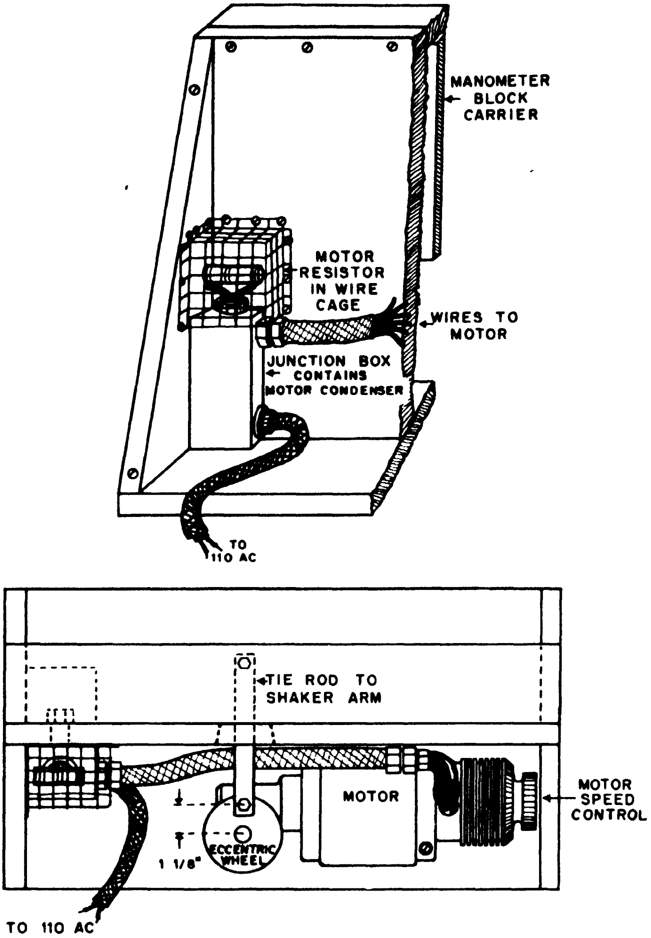

Fig. 5.23 — Plan view of shaking apparatus (below) and detail (above) of electrical supply to motor.

BIBLIOGRAPHY

1. R. Kobert, quoted by W. Autenrieth and K. H. Bauer, "Die Auffindung der Gifte und stark wirkenden Arzneistoffe," 6th ed., T. Steinkopf, Leipzig, 1943.
2. H. T. Karsner and S. P. Reimann, J. Med. Research, 39: 157 (1918).
3. J. Woroschilsky, Arb. d. Pharmakol. Inst. Dorpat, 5: 1 (1890).
4. D. E. Jackson, Am. J. Physiol., 26: 381 (1910).
5. A. M. Walker, P. A. Bott, J. Oliver, and M. C. MacDowell, Am. J. Physiol., 134: 580 (1941).
6. P. Ellinger, J. Physiol., 97: 433 (1940).
7. R. F. Pitts and W. D. Lotspeich, Am. J. Physiol., 147: 138 (1946).

8. C. K. Watanabe, J. Oliver, and T. Addis, J. Exptl. Med., 28: 359 (1918).
9. E. M. Tribe, F. G. Hopkins, and J. Barcroft, J. Physiol., 50: xi (1916).
10. M. E. Bobey, L. P. Longley, R. Dickes, J. W. Price, and J. M. Hayman, Jr., Am. J. Physiol., 139: 155 (1943).
11. A. W. Hewlett, "Pathological Physiology of Internal Diseases," 3d ed., p. 422, D. Appleton & Company, New York, 1928, quoting unpublished work of Hewlett, Gilbert, and Wickett.
12. E. Becher, Ergeb. ges. Med., 18: 51 (1933).
13. T. R. Harrison and M. F. Mason, Medicine, 16: 1 (1937).
14. P. Genaud, Compt. rend. soc. biol., 104: 550 (1930).
15. A. B. Eisenbrey, J. Exptl. Med., 14: 462 (1913).
16. C. Breedis, C. M. Flory, and J. Furth, Arch. Path., 36: 402 (1943).
17. J. P. Simonds and O. E. Hepler, Arch. Path., 39: 103 (1945).
18. D. D. Van Slyke, G. C. Linder, A. Hiller, L. Leiter, and J. F. McIntosh, J. Clin. Invest., 2: 255 (1926).
19. E. Přybil, Compt. rend. soc. biol., 102: 402 (1929).
20. Pipat, Thèse Bordeaux, 1929, quoted by Y. A. F. J. Traissac, Thèse Bordeaux, 1932.
21. H. D. Haskins, J. Biol. Chem., 2: 271 (1906).
22. J. L. Gamble, G. S. Ross, and F. F. Tisdall, J. Biol. Chem., 57: 633 (1923).
23. W. C. Hunter, Ann. Internal Med., 1: 747 (1928).
24. W. de B. MacNider, Proc. Soc. Exptl. Biol. Med., 16: 84 (1919).
25. F. B. Kingsbury, C. P. Clark, G. Williams, and A. L. Post, J. Lab. Clin. Med., 11: 981 (1926).
26. W. W. Hall and P. S. Gault, U.S. Naval Med. Bull., 38: 1 (1940).
27. A. K. Balls and W. S. Hale, J. Assoc. Offic. Agr. Chemists, 15: 483 (1932).
28. M. Dixon, "Manometric Methods," 2d ed., The Macmillan Company, New York, 1943.
29. J. B. Sumner and A. L. Dounce, J. Biol. Chem., 121: 417 (1937); 127: 439 (1939).
30. H. von Euler and K. Josephson, Ann., 452: 158 (1927).
31. H. C. Bazett and W. H. Erb, J. Pharmacol. Exptl. Therap., 49: 352 (1933).
32. J. P. Peters and D. D. Van Slyke, "Quantitative Clinical Chemistry," Vol. 2, "Methods," The Williams & Wilkins Company, Baltimore, 1932.
33. O. Schales and S. S. Schales, J. Biol. Chem., 140: 879 (1941).
34. H. E. Harrison, Proc. Soc. Exptl. Biol. Med., 49: 111 (1942).
35. L. K. Alpert, Bull. Johns Hopkins Hosp., 68: 522 (1941).
36. A. L. Albanese and V. Irby, J. Biol. Chem., 153: 583 (1944).
37. O. Folin, J. Biol. Chem., 17: 469 (1914).
38. H. Wu and S. M. Ling, Chinese J. Physiol., 1: 161 (1927).
39. S. Y. Wong, J. Biol. Chem., 55: 431 (1923).
40. D. D. Van Slyke and G. E. Cullen, J. Biol. Chem., 24: 117 (1916).
41. E. J. Conway, "Micro Diffusion Analysis and Volumetric Error," D. Van Nostrand Company, Inc., New York, 1940.
42. P. Eggleton, S. R. Elsden, and N. Gough, Biochem. J., 37: 526 (1943).
43. J. B. Sumner, J. Biol. Chem., 65: 393 (1925).
44. D. D. Van Slyke and J. A. Hawkins, J. Biol. Chem., 83: 51 (1929).
45. P. A. Shaffer and M. Somogyi, J. Biol. Chem., 100: 695 (1933).
46. N. Nelson, J. Biol. Chem., 153: 375 (1944).
47. S. B. Barker and W. H. Summerson, J. Biol. Chem., 138: 535 (1941).
48. R. H. Koeneman, J. Biol. Chem., 135: 105 (1940).
49. T. E. Friedemann and G. E. Haugen, J. Biol. Chem., 147: 415 (1943).
50. G. W. Pucher, C. C. Sherman, and H. B. Vickery, J. Biol. Chem., 113: 235 (1936).
51. L. A. Crandall, Jr., J. Biol. Chem., 133: 539 (1940).
52. L. A. Crandall, Jr., J. Biol. Chem., 138: 123 (1941).
53. O. Folin and W. Denis, J. Biol. Chem., 22: 305 (1915).

54. G. A. Snow and S. S. Zilva, Biochem. J., 37: 630 (1943).
55. J. H. Roe and M. J. Oesterling, J. Biol. Chem., 152: 511 (1944).
56. G. Medes, Biochem. J., 29: 2251 (1935).
57. O. Folin and H. Wu, J. Biol. Chem., 38: 81 (1919).
58. H. Irving and J. C. Forbes, J. Lab. Clin. Med., 20: 316 (1934).
59. F. C. Koch and T. L. McMeekin, J. Am. Chem. Soc., 46: 2066 (1929).
60. O. Folin and A. Svedberg, J. Biol. Chem., 88: 77 (1930).
61. O. Folin and J. B. Sumner, quoted by J. B. Sumner and G. F. Somers, "Laboratory Experiments in Biological Chemistry," Academic Press, New York, 1944.
62. O. Folin, J. Biol. Chem., 17: 475 (1914).
63. S. R. Benedict and J. A. Behre, J. Biol. Chem., 92: 161 (1931).
64. O. Folin, J. Biol. Chem., 101: 111 (1933); 106: 311 (1934).
65. M. E. Blauch and F. C. Koch, J. Biol. Chem., 130: 443 (1939).
66. E. G. Frame, J. A. Russell, and A. E. Wilhelmi, J. Biol. Chem., 149: 255 (1943).
67. J. A. Russell, J. Biol. Chem., 156: 467 (1944).
68. O. Folin and H. Wu, J. Biol. Chem., 41: 367 (1920).
69. O. Folin and H. Malmros, J. Biol. Chem., 83: 115 (1929).
70. M. Somogyi, J. Biol. Chem., 80: 733 (1928); 87: 339 (1930).
71. S. R. Benedict, J. Biol. Chem., 76: 457 (1928); 92: 141 (1932).
72. J. B. Sumner and E. B. Sisler, Arch. Biochem., 4: 333 (1944).
73. O. Schales and S. S. Schales, Arch. Biochem., 8: 285 (1945).
74. J. H. Roe and B. S. Kahn, J. Biol. Chem., 81: 1 (1929).
75. F. Rappaport and D. Rappaport, Microchem., 15: 107 (1934).
76. C. H. Fiske and Y. Subbarow, J. Biol. Chem., 66: 375 (1925).
77. S. R. Benedict and R. C. Theis, J. Biol. Chem., 61: 63 (1924).
78. J. B. Sumner, Science, 100: 413 (1944).
79. N. W. Rakestraw, J. Biol. Chem., 56: 109 (1923).
80. R. C. Theis and S. R. Benedict, J. Biol. Chem., 61: 67 (1924).
81. P. R. Roen, J. Urol., 51: 110 (1944).
82. D. M. Greenberg, J. Biol.Chem., 82: 545 (1929).
83. J. M. Looney and A. I. Walsh, J. Biol. Chem., 130: 635 (1939).
84. P. E. Howe, J. Biol. Chem., 49: 109 (1921).
85. M. Reiner, "Manual of Clinical Chemistry," Interscience Publishers, Inc., New York, 1941.
86. H. A. Campbell, W. K. Smith, W. L. Roberts, and K. P. Link, J. Biol. Chem., 138: 1 (1941).
87. W. R. Bloor, K. F. Pelkan, and D. M. Allen, J. Biol. Chem., 52: 191 (1922).
88. F. E. Kelsey, J. Biol. Chem., 127: 15 (1939).
89. J. C. Forbes and H. Irving, J. Lab. Clin. Med., 16: 909 (1931).
90. W. R. Bloor, J. Biol. Chem., 82: 273 (1929).
91. M. Yasuda, J. Biol. Chem., 92: 303 (1931).
92. S. M. Rosenthal and E. C. White, J. Am. Med. Assoc., 84: 1112 (1925).

Chapter 6

TOXICITY FOLLOWING THE PARENTERAL ADMINISTRATION OF CERTAIN SOLUBLE URANIUM COMPOUNDS

By Frances L. Haven and Harold C. Hodge*

1. INTRODUCTION

This chapter presents a summary of the toxicity studies on animals that received various uranium compounds parenterally. The data cited here were collected by a number of investigators and for a variety of reasons. In most cases the compounds were administered intraperitoneally. In a few cases the intravenous route was employed. The studies of toxicity in this chapter are really studies of mortality, and in most cases they are studies of rats.

Many data that might have been included in this chapter are given in other chapters. To avoid duplication, such data are not repeated here. However, in the chapters on mechanism and the characteristics of uranium poisoning (Chaps. 5, 12, 14, 15) will be found considerable additional information on the subject of parenteral toxicity. These data were gathered in studies devoted to therapeutic procedures against uranium poisoning, studies of functional changes, studies of the effects of various diets on the course of poisoning, studies of sensitive methods for the detection of the earliest signs of poisoning, and studies of tolerance. In general, the mortality data in these studies were in line with the data reported in this chapter.

A review of the literature has given the information on dogs and rabbits shown in Table 6.1. MacNider[1,2,3] has studied a large series of dogs administered doses of uranium nitrate subcutaneously. The fatal dose for the average dog is apparently somewhat greater than 2 mg U/kg. Recently, Donnelly and Holman[4] gave 2.4 mg U/kg subcutaneously to 13 dogs and found a mortality of 92 per cent. The only work in which intravenous administration was used is the study of Gustafson *et al.*,[5] in which five out of five dogs died following the administration of 3.2 mg U/kg. The work on rabbits was performed by Hunter[6] using both intravenous and subcutaneous routes. Only a rough estimate of the fatal dose for the average rabbit is possible from his data, but it appears that about 0.4 mg of uranium intravenously and 0.7 mg of uranium subcutaneously would kill the average rabbit. Unfortunately, Hunter did not express his dose in terms of milligrams per kilogram of body weight. However, in several instances he referred to rabbits weighing 1 to 3 kg.

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*Work done by Harold C. Hodge, Frances L. Haven, Elliott Maynard, James Orcutt, Jessie Miller, Isabel Thomas, and Virginia Edwards.

Table 6.1 — Mortality in Dogs and Rabbits after Parenteral Administration of Uranyl Nitrate Hexahydrate

Investigator	Date	Route	Mg U/kg	No. of deaths per no. of animals
		Dogs		
MacNider	1929	Subcutaneous	0.9	8/41
MacNider	1936	Subcutaneous	0.9	10/38
MacNider	1936	Subcutaneous	0.9	6/18
MacNider	1934-1935	Subcutaneous	1.9	16/34
MacNider	1936	Subcutaneous	1.9	13/32
MacNider	1936	Subcutaneous	1.9	8/24
Donnelly and Holman	1942	Subcutaneous	2.4	12/13
Gustafson *et al.*	1944	Intravenous	3.2	5/5
			LD_{50} = 2+	
		Rabbits		
			Mg U/rabbit*	
Hunter	1928	Intravenous	0.2	1/24
Hunter	1928	Intravenous	0.5	4/4
Hunter	1928	Subcutaneous	0.5	3/31
Hunter	1928	Subcutaneous	0.9	9/10
Hunter	1928	Intravenous	0.9	2/2
		LD_{50}(intravenous)	= 0.4+	
		LD_{50}(subcutaneous)	= 0.7+	

* Rabbits weighed 1 to 3 kg.

The data in the following pages are grouped under two main heads:

Toxicity Following Intraperitoneal Administration. In this section the studies of rats are divided into two main divisions: (1) studies in which doses large enough to produce mortality in 24 to 48 hr were used, and (2) those in which doses produced mortality in 14 to 21 days. The data on mice included studies on albino and C_3H mice.

Toxicity Following Intravenous Administration. Preliminary data on rabbits, guinea pigs, and mice are presented.

2. THE MORTALITY FOLLOWING INTRAPERITONEAL ADMINISTRATION

2.1 Mortality in 24 Hr, Rats. One of the toxic constants that may be determined is the acute toxicity, i.e., the 24-hr mortality following the administration via peritoneal, oral, or other routes. The albino rat is frequently used as a test animal in such studies.

The acute toxicity of three uranium compounds has been measured using sufficiently large numbers of rats to establish dosage-mortality curves and to give a statistically determined LD_{50}.

The general procedure in all these studies has been approximately the same. Wistar strain rats were used throughout. Groups of a few rats, i.e., five or six, were given intraperitoneal doses of widely varying amounts of the compounds in aqueous solutions. The numbers of animals dead at the end of 24 hr and at 48 hr were recorded. In a few cases the surviving animals were watched for additional deaths during the following period of 2 weeks. However, for the most part these experiments were performed for the sole purpose of determining the 24- and 48-hr mortality. The rats were not observed for longer periods. When the region of dosage that seemed to kill about half the animals was discovered, much larger groups of rats, 15 to 25 per group, were tested at closely spaced doses near that which gave a 50 per cent mortality. Additional groups were tested until sufficient data were available for a statistical analysis. The treatment of the data by the statistics department followed the method for calculating the dosage-mortality relationship given by Bliss.[7] In this method the percentage mortality is converted to probits,* and these derived numbers are plotted against the logarithm of the dosage. In many studies the probit-logarithm dosage curve was shown to be a straight line in the region between 20 and 80 per cent mortality.

(a) Uranyl Nitrate Hexahydrate. As an illustration of the sort of data obtained, Fig. 6.1 shows the percentage mortality in 300- to 400-g male rats following the administration of uranyl nitrate hexahydrate given intraperitoneally as a 10 per cent aqueous solution. The percentage mortality-dosage curve for the first 24 hr is a typical sigmoid curve. Placed somewhat above the curve for mortality within 24 hr are the points that indicate the respective mortalities within 48 hr. As may be seen, these are all 80 per cent or greater.

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*"Probit" is a coined word from probability unit. The conversion of percentage mortality to a probability scale in which the standard deviation is the unit of measurement from the mean transforms the percentage mortality curve to a straight line when dosage is plotted as the logarithm, base 10.

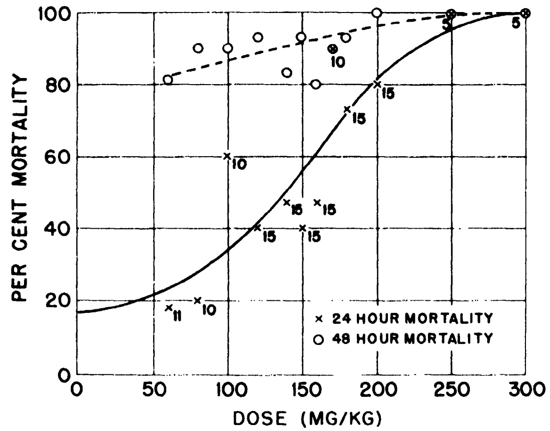

Fig. 6.1 —The percentage mortality is plotted against the dose in milligrams per kilogram for 300- to 400-g male rats given a 10 per cent aqueous solution of $UO_2(NO_3)_2.6H_2O$ intraperitoneally. The figures beside the points indicate the number of animals per point for the 24-hr mortality.

It is evident from the variation in the mortality above and below the S curve for the 24-hr mortality that considerable doubt exists as to exactly where this line should be placed. Consequently, interpolating the dosage corresponding to a 50 per cent mortality involves considerable uncertainty. It would appear from this curve that a dose of the order of 140 to 145 mg/kg would produce a 50 per cent mortality. The device referred to above of employing probits attempts to get away from the difficulty of judging the exact position of a complex curve, such as the one shown, by providing data that lie along a straight line. Almost without exception, the mortality data that follow are given in the figures in terms of probits.

Male Rats, 50 to 100 g. Groups of young male rats were given doses varying from 200 to 400 mg/kg. The results shown in Fig. 6.2 fairly closely approximate a straight line for the 24-hr values. The LD_{50} for the 24-hr mortality is of the order of 305 mg/kg. The calculated dosage interval that would include such a value 95 times in 100 is from

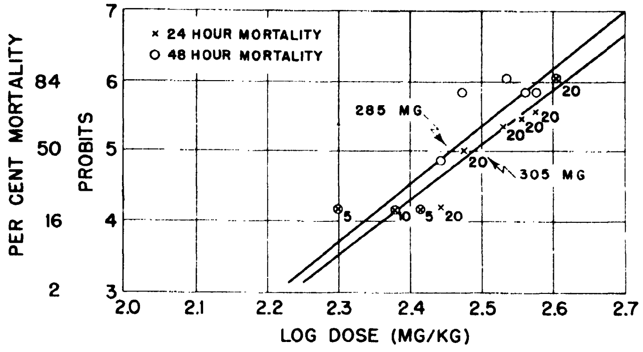

Fig. 6.2—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 50- to 100-g male rats given a 10 per cent aqueous solution of $UO_2(NO_3)_2.6H_2O$ intraperitoneally.

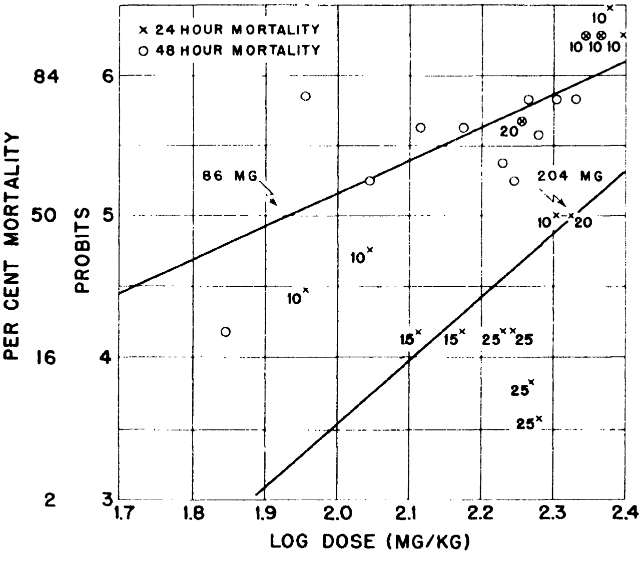

Fig. 6.3—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 200- to 300-g male rats given a 10 per cent aqueous solution of $UO_2(NO_3)_2.6H_2O$ intraperitoneally.

287 to 324 mg/kg. The 48-hr LD_{50} is about 285 mg/kg with a range of 268 to 304.

Male Rats, 200 to 300 g. A total of 245 male rats weighing from 200 to 300 g were divided into groups and given doses of 70 to 250 mg/kg. The results as shown in Fig. 6.3 were scattered. The LD_{50} for the 24-hr period as calculated from 16 doses is of the order of 204 mg/kg. The range is 167 to 250. The LD_{50} for mortality after 48 hr is about 86 mg/kg with a range of 53 to 138 mg/kg.

Male Rats, 300 to 400 g. One hundred forty-one male rats weighing 300 to 400 g were divided into groups and given doses varying from 60 to 350 mg/kg. As shown in Fig. 6.4, the trend line for the 24-hr period is fairly well located. The scattering of points is not so great as in the preceding experiment. The LD_{50} for 24 hr is about 128 mg/kg based on 12 different doses; the range is 120 to 136. The LD_{50} for 48-hr mortality could not be determined from the data shown in Fig. 6.4.

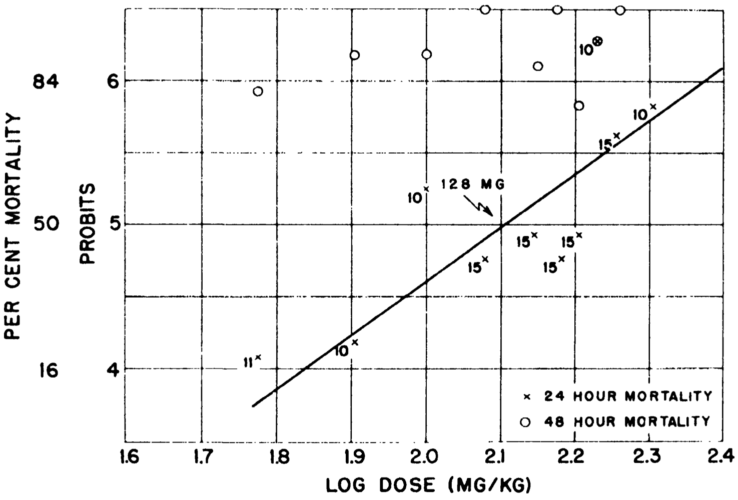

Fig. 6.4—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 300- to 400-g male rats given a 10 per cent aqueous solution of $UO_2(NO_3)_2.6H_2O$ intraperitoneally.

Increase in Toxic Effect with Age. In Fig. 6.5 are placed the three lines just described for the probits vs. logarithms of dosages. An amazing increase in toxicity of uranyl nitrate hexahydrate with increasing age of rats is plainly evident. The adult rats weighing 300 to 400 g required a little over 100 mg/kg to kill the average rat (left-hand line). The young adult rats weighing 200 to 300 g required about 200 mg/kg (center line), whereas the young rats weighing 50 to 100 g required just over 300 mg/kg (right-hand line). There was a marked increase in toxicity of uranyl nitrate hexahydrate with increasing age.

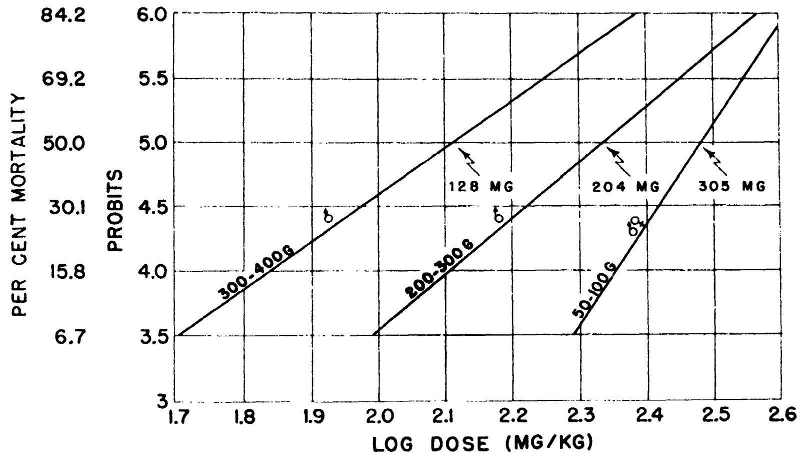

Fig. 6.5—The percentage mortality in 24 hr and probits are plotted against the logarithm of the dose in milligrams per kilogram for rats given a 10 per cent aqueous solution of $UO_2(NO_3)_2.6H_2O$ intraperitoneally. The arrows indicate the approximate LD_{50} in milligrams per kilogram for each group of rats.

Female Rats, 200 to 300 g. Two hundred fifty female rats weighing from 200 to 300 g were divided into groups and given doses of uranyl nitrate hexahydrate, ranging from 70 to 400 mg/kg. As shown in Fig. 6.6, there was a widespread scatter of the mortality data. The LD_{50} for the 24-hr period was of the order of 135 mg/kg; the range was 120 to 152. The LD_{50} for the 48-hr period was about 53 mg/kg; the range was 22 to 126.

The LD_{50} for the 24-hr period of these adult females (135 mg/kg) compared favorably with that for the 300- to 400-g males (128 mg/kg). On the other hand, the LD_{50} for these females is quite unlike that for males of the same weight (135 for females, 204 for males). These data indicate that the relationship of toxicity is not with body weight but with age, since 200- to 300-g female rats correspond in age to 300- to 400-g male rats. Although insufficient data are available to give a conclusive statement on this point, it might be noted that tests on young female rats, 50 to 100 g, gave indications that the LD_{50} at this age would be about equal to that for male rats of the same age, namely, of the order of 300 mg/kg.

Toxic Symptoms. The rats showed with fair regularity a group of toxic effects. Diarrhea was frequent, as was a red exudate about the nose. Swollen and purple tongues were frequently seen. Bloody fluid was found in most of the abdominal cavities of rats dying within 24 hr. One of the striking findings (Fig. 6.7) was the appearance in the wall of the small intestine of small hemorrhages ranging from 1 to 5 or 6 mm in diameter, roughly circular in outline. Hemorrhages were also seen here and there in the connective tissue surrounding the spleen and stomach.

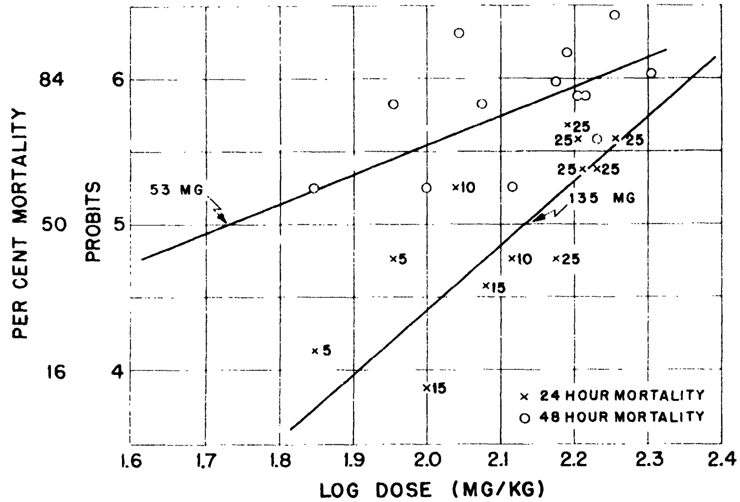

Fig. 6.6 — The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 200- to 300-g female rats given a 10 per cent aqueous solution of $UO_2(NO_3)_2.6H_2O$ intraperitoneally.

(b) UO_2F_2. Somewhat less extensive experiments were carried out to establish the acute toxicity of UO_2F_2 in rats. Three experiments were performed, the first on males and females weighing 50 to 100 g, the second on males and females weighing 150 to 200 g, and the third on males weighing 300 to 400 g.

Male and Female Rats, 50 to 100 g. Ninety male and female rats weighing 50 to 100 g were divided into groups and given doses varying from 40 to 140 mg/kg. As seen in Fig. 6.8, the mortality as probits

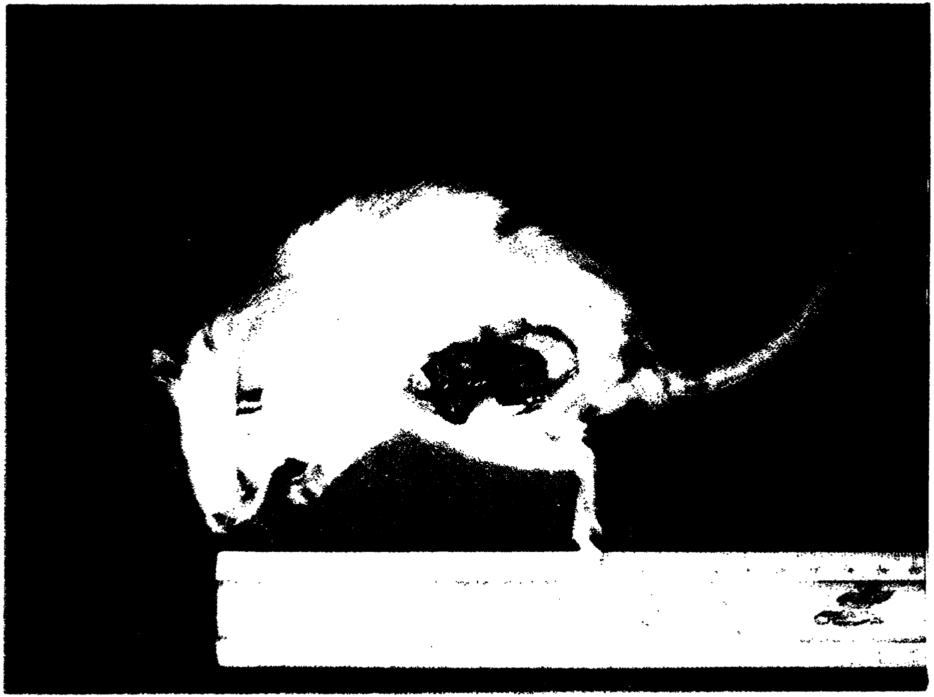

Fig. 6.7—Photograph showing hemorrhages in the wall of the small intestine of rat given acute intraperitoneal doses of uranyl nitrate.

plotted against the logarithm of the dosage did not reveal any abnormal scattering of the data. The LD_{50} for the 24-hr period was of the order of 78 mg/kg; the range was 65 to 94. The LD_{50} for the 48-hr period was of the order of 60 mg/kg; range, 42 to 84.

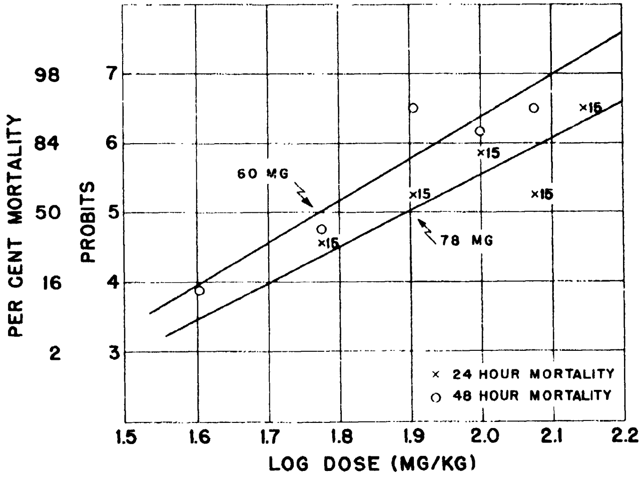

Fig. 6.8 —The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 50- to 100-g rats of both sexes given a 2 per cent aqueous solution of UO_2F_2 intraperitoneally.

Male and Female Rats, 150 to 200 g. One hundred sixty-five male and female rats weighing 150 to 200 g were divided into groups and given doses of UO_2F_2 ranging from 50 to 120 mg/kg. As shown in Fig. 6.9, there was a considerable scatter of the data. The LD_{50} for the 24-hr period was about 87 mg/kg; the range was 79 to 95. The scatter was so large for the 48-hr period that it is difficult to establish an LD_{50} for this interval.

Male Rats, 300 to 400 g. One hundred sixty male rats weighing 300 to 400 g were divided into groups of 10 to 20 each and given doses of UO_2F_2 ranging from 5 to 100 mg/kg. Relatively slight variation was exhibited by the mortalities plotted in Fig. 6.10. The LD_{50} was of the order of 40 mg/kg with a range of 36 to 44. Peculiarly, the LD_{50} for the 48-hr period was about the same, namely, 33 mg/kg; range, 28 to 38.

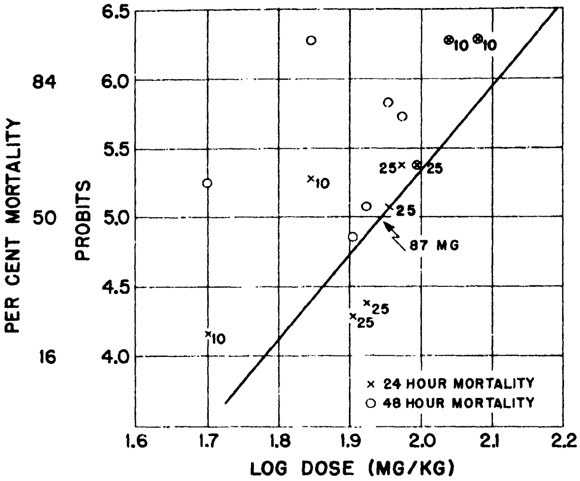

Fig. 6.9—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 150- to 200-g rats of both sexes given a 2 per cent aqueous solution of UO_2F_2 intraperitoneally.

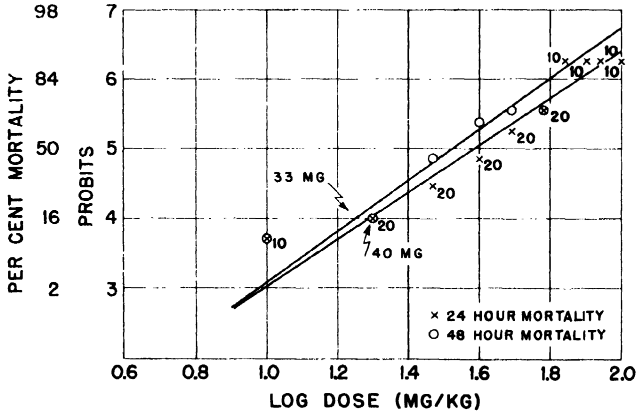

Fig. 6.10—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 300- to 400-g male rats given a 2 per cent aqueous solution of UO_2F_2 intraperitoneally.

Symptoms. The signs of acute toxicity to uranyl fluoride were very much like those seen after acute doses of uranyl nitrate. Diarrhea frequently developed and the animals became dull, restless, and comatose. Rats that received the higher doses often died promptly, i.e., within 30 min to 4 hr. In certain groups those that survived 72 hr were observed for 2 to 3 weeks, in which time they appeared to regain their appetite and to be relatively healthy. In the animals that died, bloody fluid was found in the abdominal cavities. The hemorrhagic areas described in the small intestine following acute doses of uranyl nitrate were also seen. The observation was made that these areas did not occur in the large intestine of rats receiving UO_2F_2.

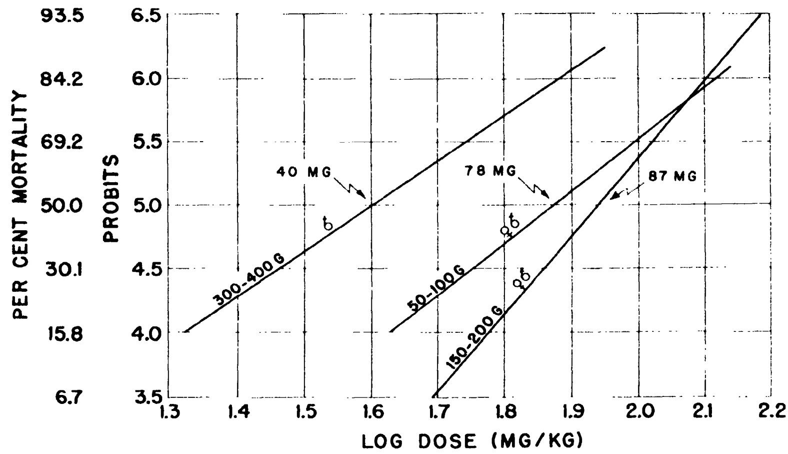

Fig. 6.11—The percentage mortality in 24 hr and probits are plotted against the logarithm of the dose in milligrams per kilogram for rats given a 2 per cent aqueous solution of UO_2F_2 intraperitoneally.

The same increase in toxicity with age was found for this compound as for uranyl nitrate (Fig. 6.11). The LD_{50}'s for the male and female rats weighing 50 to 100 g were so nearly alike that there was probably no difference. The LD_{50} for a 24-hr period in 150- to 200-g males and females was of the order of 78 to 87. In contrast, the LD_{50} for the 24-hr interval of 300- to 400-g male rats was approximately half the value for the younger rats, namely, 40 mg/kg.

It is apparent that UO_2F_2 is much more toxic than uranyl nitrate for a given weight group (compare Fig. 6.11 with Fig. 6.5).

(c) UCl_4. The studies of the acute toxicity of UCl_4 were complicated by an attempt to compare the effects of a partially neutralized solution with those of a simple aqueous solution. The partially neutralized solution contained 5 per cent (i.e., approximately 0.1M) UCl_4 in a 1M sodium acetate solution. The pH of 4.9 that was thus obtained is near the limit of solubility of UCl_4. A total of 815 rats was used in this study, divided into five groups according to their weight and sex.

An additional study was carried out using 465 rats with a 10 per cent aqueous solution of UCl_4 having a pH of 1.3 when buffered and a pH of 3.6 when buffered with 1M sodium acetate.

Male and Female Rats, 50 to 100 g. Two hundred male and female rats weighing 50 to 100 g were divided into groups of 10 to 20 each and given 400 to 750 mg UCl_4/kg in sodium acetate solution. As shown in Fig. 6.12, a close dependence of mortality on dosage was found. The LD_{50} for a 24-hr interval was of the order of 615 mg/kg, with a range of 598 to 633. The 48-hr LD_{50} was about 480 mg/kg, with a range of 454 to 508.

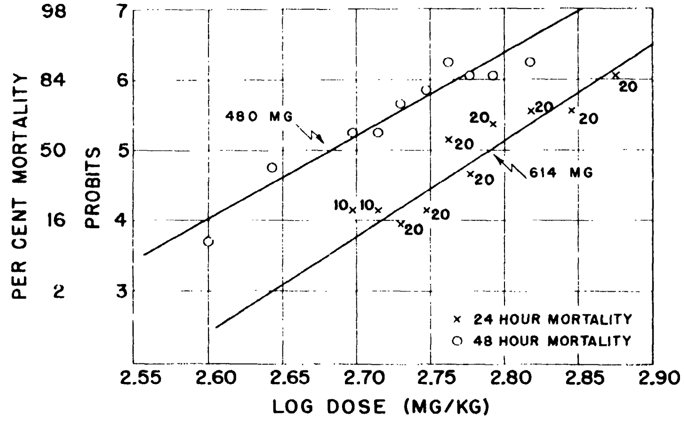

Fig. 6.12 — The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 50- to 100-g rats of both sexes given intraperitoneally a 5 per cent aqueous solution of UCl_4 in 1M sodium acetate at pH 4.9.

Male Rats, 100 to 200 g. One hundred twenty male rats weighing 100 to 200 g were given various doses ranging from 250 to 600 mg UCl_4/kg in sodium acetate solution. The mortalities as shown in Fig. 6.13 fell about a straight line when probits were plotted against logarithms of dosage. The LD_{50} was of the order of 434 mg/kg; the range was 411 to 458. The LD_{50} for the 48-hr interval was of the order of 345 mg/kg; range 304 to 392.

Male Rats, 300 to 400 g. One hundred seventy-five male rats weighing 300 to 400 g were injected with various doses ranging from 150 to

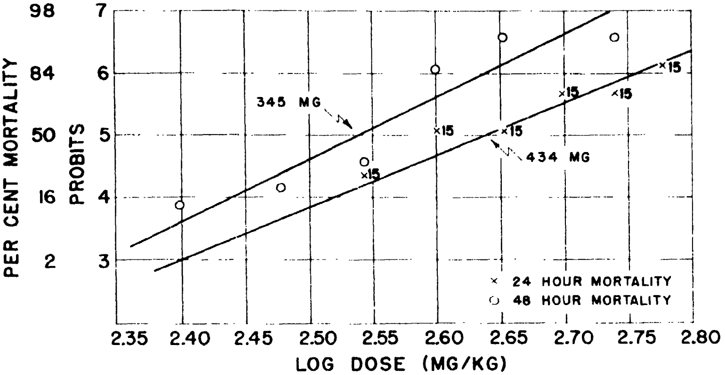

Fig. 6.13—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 100- to 200-g male rats given intraperitoneally a 5 per cent aqueous solution of UCl_4 in 1M sodium acetate at pH 4.9.

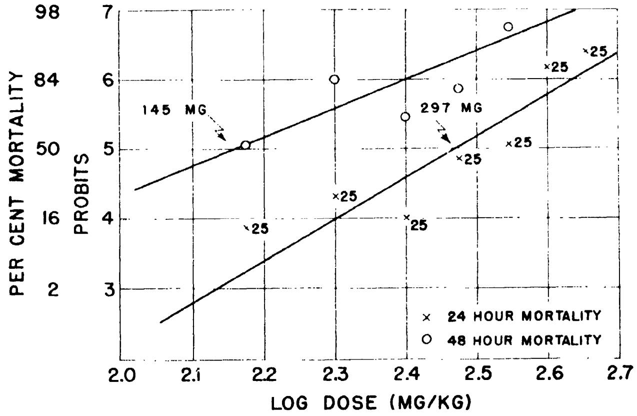

Fig. 6.14—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 300- to 400-g male rats given intraperitoneally a 10 per cent aqueous solution of UCl_4 in 1M sodium acetate at pH 3.6.

450 mg UCl_4/kg in sodium acetate solution. Figure 6.14 shows a close dependence of mortality on dosage. The LD_{50} was of the order of 298 mg/kg; range 260 to 341. The LD_{50} for the 48-hr period was about 145; range 89 to 235.

Female Rats, 100 to 200 g. One hundred twenty female rats weighing 100 to 200 g were given various doses ranging from 250 to 600 mg UCl_4/kg in sodium acetate solution. A close dependence of mortality on dosage is shown in Fig. 6.15. The LD_{50} was of the order of 368 mg/kg; range 327 to 415. The 48-hr LD_{50} was about 253; range 136 to 461.

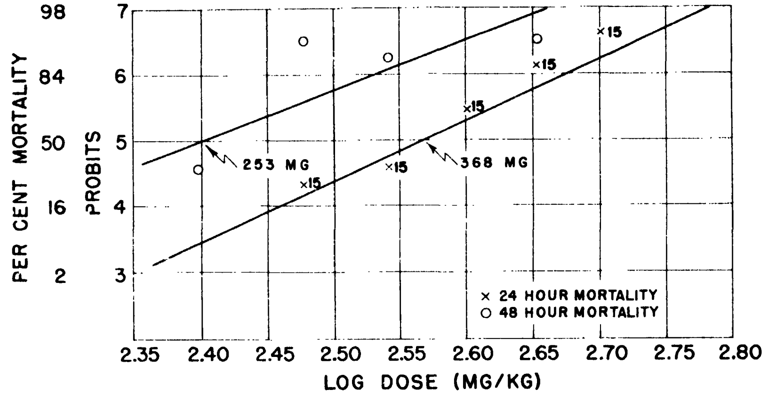

Fig. 6.15—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 100- to 200-g female rats given intraperitoneally a 5 per cent aqueous solution of UCl_4 in 1M sodium acetate at pH 4.9.

Female Rats, 200 to 300 g. Two hundred female rats weighing 200 to 300 g were given various doses ranging from 170 to 450 mg UCl_4/kg in sodium acetate solution. Figure 6.16 shows the dependence of mortality on dosage. The LD_{50} at 24 hr was 350 mg/kg; range 330 to 371. The LD_{50} at 48 hr was about 227; range 198 to 259.

Comparing the mortalities of three groups—(1) rats of both sexes weighing 50 to 100 g, (2) male rats weighing 100 to 200 g, and (3) male rats weighing 300 to 400 g—a marked increase in toxicity with increase in age was observed. There was some evidence of a similar increase in toxicity in the case of the females. Thus, the LD_{50} was of the same order for 100- to 200-g females as for 200- to 300-g females, although both these groups required only a little more than half as much UCl_4 as killed the average female rat of weight 50 to 100 g. It was evident that UCl_4 was much less toxic than uranyl nitrate or UO_2F_2 for both male and female rats.

Male and Female Rats, 50 to 100 g. One hundred sixty-five male and female rats weighing 50 to 100 g were given various doses ranging

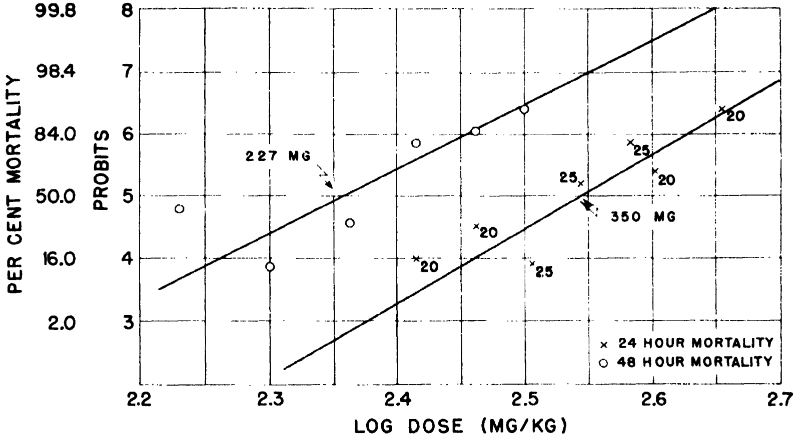

Fig. 6.16—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 200- to 300-g female rats given intraperitoneally a 5 per cent aqueous solution of UCl_4 in 1M sodium acetate at pH 4.9.

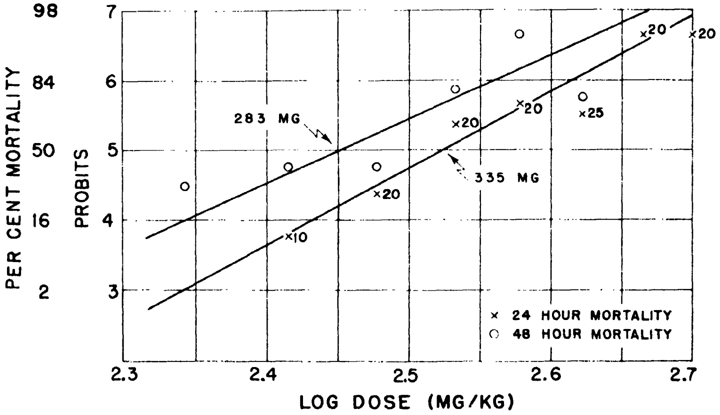

Fig. 6.17—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 50- to 100-g rats of both sexes given a 10 per cent aqueous solution (pH 1.3) of UCl_4 intraperitoneally.

from 220 to 500 mg UCl_4/kg as a 10 per cent aqueous solution. A close dependence of mortality on dosage is shown in Fig. 6.17. The LD_{50} was of the order of 335 mg/kg; range 314 to 358. The 48-hr LD_{50} was about 283; range 240 to 333.

Female Rats, 200 to 300 g. Three hundred female rats weighing 200 to 300 g were given doses ranging from 100 to 350 mg UCl_4/kg as a 10 per cent aqueous solution. The various points fell closely about the line shown in Fig. 6.18. The LD_{50} was of the order of 190 mg/kg; range 176 to 204. The 48-hr LD_{50} was about 111 mg/kg; range 99 to 124.

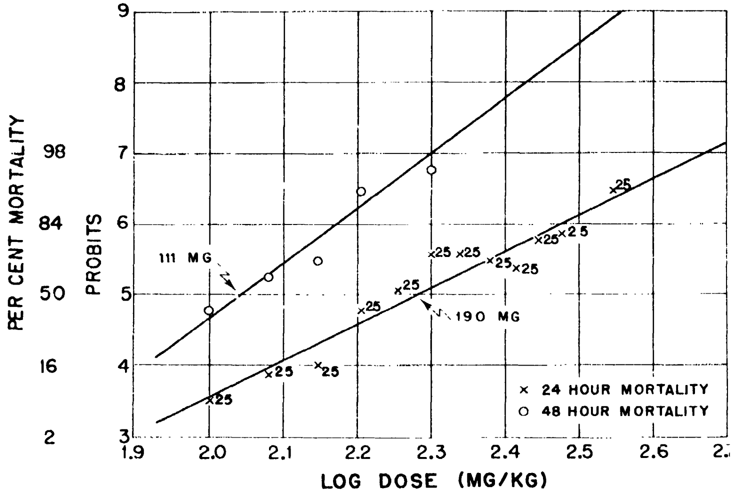

Fig. 6.18—The percentage mortality and probits are plotted against the logarithm of the dose in milligrams per kilogram for 200- to 300-g female rats given a 10 per cent aqueous solution (pH 1.3) of UCl_4 intraperitoneally.

The 10 per cent aqueous solution of UCl_4 was considerably more toxic than the 5 per cent UCl_4 solution in sodium acetate. For the young rats, 50 to 100 g, only half as much unbuffered UCl_4 was required as of the sodium acetate buffered solution. A similar difference, although not quite so large in magnitude, was noted for the older female rats.

Since the buffered solution was less toxic, it was natural to inquire whether the acidity was responsible for the extra toxicity. A series of rats, 10 to each group, were given doses ranging from 0.18 to 0.26 ml of a 0.01N solution of HCl_4 having a pH of 1.4. None of these doses caused any mortality. Consequently it was not the acid alone that made the difference in toxicity of the two UCl_4 solutions.

The difference in the toxicity between the aqueous solution and the sodium acetate solution was perhaps related to the concentration of uranium: The 10 per cent solution was more toxic than the 5 per cent solution. It is interesting to note that the increase in toxicity of the 10 per cent solution was uniform when the LD_{50}'s are compared for the 50- to 100-g male and female rats and the 200- to 300-g female rats. (See the accompanying table.)

		LD_{50} of UCl_4	
Sex	Weight, g	5% in NaAc	10% in water
Male and female	50 – 100	615	335
Female	200 – 300	350	190

The toxicity of the 10 per cent solution was 1.83 times as great as that of the 5 per cent solution in 50- to 100-g rats, and 1.84 times as great in 200- to 300-g rats. This was an extraordinary degree of consistency and indicated that concentration may be an important factor.

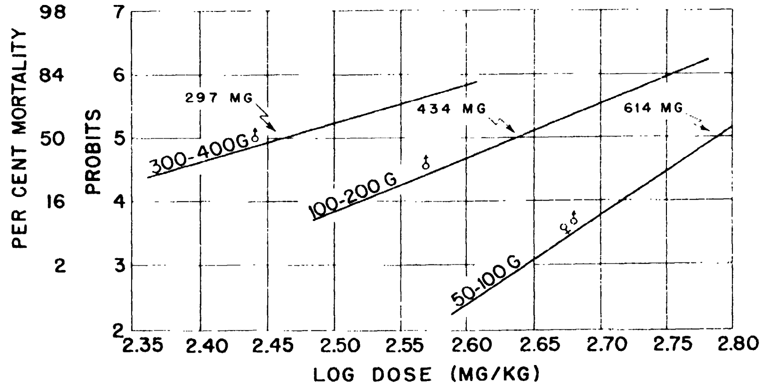

Fig. 6.19 — The percentage mortality in 24 hr and probits are plotted against the logarithm of the dose in milligrams per kilogram for rats of different body weights given intraperitoneally a 5 per cent solution of UCl_4 in 1M sodium acetate at pH 4.9.

In Fig. 6.19 the acute toxicity of UCl_4 is compared for three groups of rats, namely, 50- to 100-g male and female rats, 100- to 200-g males, and 300- to 400-g males. The corresponding LD_{50}'s were 614, 434, and 297 mg/kg. The increase in toxicity with increase in age was similar to that observed for animals treated with uranyl nitrate and those treated with UO_2F_2.

Symptoms. The symptoms of acute toxicity were very much like those seen in rats poisoned with uranyl nitrate or UO_2F_2. Diarrhea was a common symptom; red incrustation around the nose was frequently observed. On autopsy the abdominal cavity contained considerable bloody fluid. The livers and spleens frequently appeared gray, as if partially cooked. Violent convulsions were sometimes observed just before death. Animals that survived for 72 hr appeared to show signs of recovery, and within a week were superficially normal.

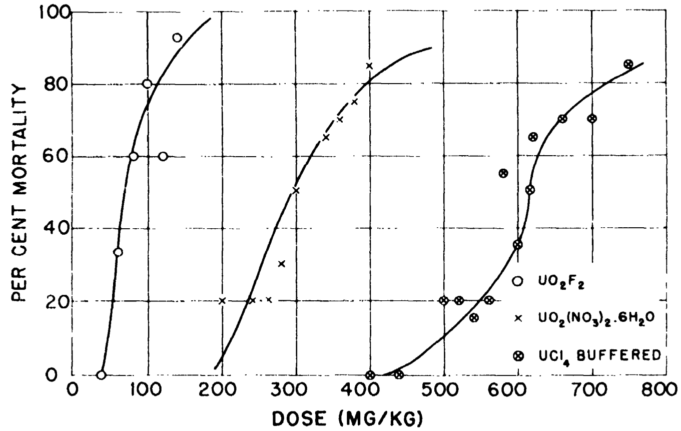

Fig. 6.20—The percentage mortality in 24 hr is plotted against the dose in milligrams per kilogram for 50- to 100-g rats of both sexes given intraperitoneal doses of uranium compounds.

(d) The Comparative Acute Toxicity of UO_2F_2, Uranyl Nitrate, and UCl_4. In Fig. 6.20 are shown the typical S-shaped curves of percentage mortality vs. dosage for solutions of UO_2F_2, uranyl nitrate, and UCl_4, the latter buffered in sodium acetate, when each was administered to 50- to 100-g male and female rats. From Fig. 6.21, in which the respective probit values are plotted against the logarithm of the dosage, the approximate LD_{50}'s were as follows: UO_2F_2, 78; uranyl nitrate hexahydrate, 305; UCl_4, 614 mg/kg. The LD_{50} for the unbuffered 10 per cent UCl_4 solution in water (335 mg/kg) was approximately the same as that for uranyl nitrate.

The high toxicity of the UO_2F_2, in part at least, was related to its content of fluoride ion. Results reported in Part II (Chap. 17) show that the acute toxicity of NaF when measured by comparable procedures was of the same order of magnitude as the ones dealt with here.

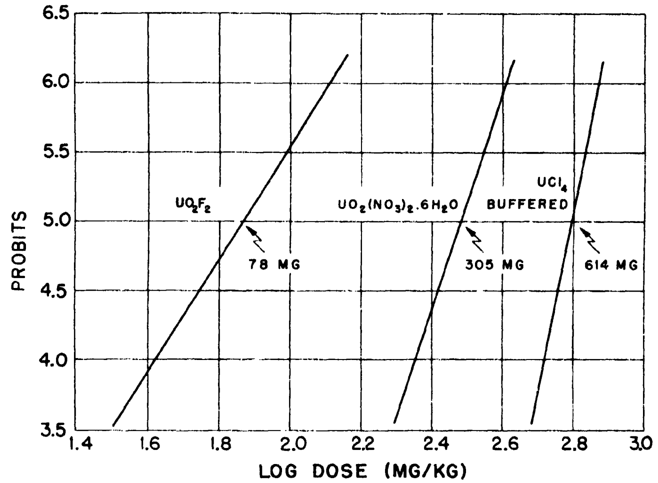

Fig. 6.21 —The 24-hr mortality expressed as probits is plotted against the logarithm of the intraperitoneal dose in milligrams per kilogram for 50- to 100-g rats of both sexes given aqueous solutions of UO_2F_2, $UO_2(NO_3)_2.6H_2O$, and UCl_4 (buffered), respectively.

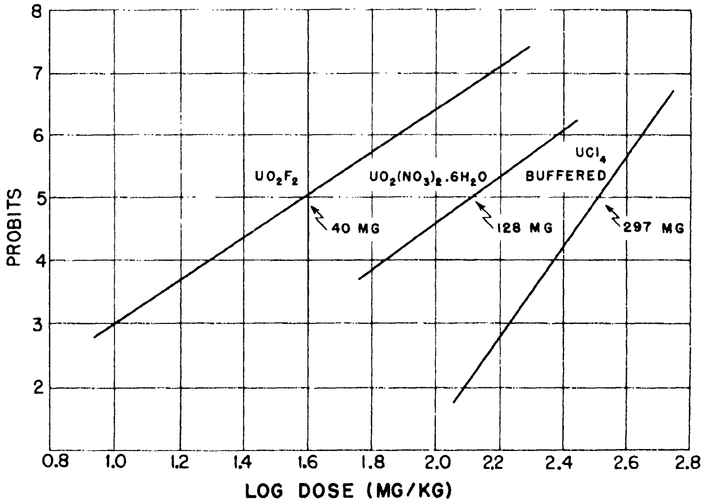

Fig. 6.22 —The probits for the 24-hr mortality are plotted against the log dose in milligrams per kilogram for 300- to 400-g male rats given intraperitoneal doses of uranium compounds. See Fig. 6.9 for UO_2F_2 data, Fig. 6.4 for $UO_2(NO_3)_2.6H_2O$ data, and Fig. 6.13 for UCl_4 (buffered) data.

Thus the LD_{50} of sodium fluoride for 24-hr mortality was 47 mg/kg for 100- to 200-g female rats, and 24 mg/kg for 200- to 300-g female rats.

It is possible to compare the toxicities of the three compounds also in the LD_{50} values as obtained for 300- to 400-g male rats. In Fig. 6.22 this comparison is illustrated. The respective LD_{50}'s were as follows: UO_2F_2, 40; uranyl nitrate hexahydrate, 128; UCl_4, 297 mg/kg. There was a remarkable regularity of increase of toxicities in Figs. 6.21 and 6.22. For 50- to 100-g male and female rats (Fig. 6.21) LD_{50}'s for UO_2F_2, uranyl nitrate, and UCl_4 stand in the ratios of 1 : 3.9 : 7.9. For 300- to 400-g male rats (Fig. 6.22) the respective values stand in the ratios of 1 : 3.2 : 7.4. The increase in toxicity from compound to compound had a high degree of regularity whether measured in young or in old rats.

(e) Summary. 1. In rats the acute toxicity of uranyl nitrate hexahydrate administered as aqueous solutions intraperitoneally gave the following data:

Sex	Weight, g	LD_{50}/24 hr, mg/kg	Reference
Male	50 – 100	305	Fig. 6.2
Male	200 – 300	204	Fig. 6.3
Male	300 – 400	128	Fig. 6.4
Female	200 – 300	135	Fig. 6.6

A striking increase in toxicity with increasing age was thus established.

2. Solutions of UO_2F_2 were given intraperitoneally to a large number of rats. Approximate LD_{50}'s for the 24-hr mortality were as follows:

Sex	Weight, g	LD_{50}, mg/kg	Reference
Male and female	50 – 100	78	Fig. 6.8
Male and female	150 – 200	87	Fig. 6.9
Male	300 – 400	40	Fig. 6.10

Uranyl fluoride is more toxic to old rats than to young ones. Uranyl fluoride is more toxic than uranyl nitrate.

3. The acute toxicity of UCl_4 administered as a 10 per cent aqueous solution gave the following data:

Sex	Weight, g	LD_{50}/24 hr, mg/kg	Reference
Male and female	50 – 100	335	Fig. 6.17
Female	200 – 300	190	Fig. 6.18

When the UCl_4 was administered in sodium acetate buffer, the following values were found:

Sex	Weight, g	LD_{50}/24 hr, mg/kg
Male and female	50 – 100	615
Male	100 – 200	434
Male	300 – 400	297
Female	100 – 200	368
Female	200 – 300	350

The toxicity increased with increasing age. A 10 per cent aqueous solution of UCl_4 was more toxic than a 5 per cent solution in sodium acetate.

2.2 Mortality within 14 to 21 Days, Rats. The rats used in the following studies were young and adult albinos that were housed in wire cages in groups of five or fewer. They were kept on a diet of Purina Fox Chow and received food and water ad lib. To standardize the injection procedure, the animals were injected always at the same time of day — in the early afternoon. The rats were weighed for several days prior to injection and daily thereafter until death or gain in weight occurred. With young rats the experiment was terminated in 14 days, with adult rats at 21 days. The peak mortality occurred at approximately the fifth day after injection in the case of young rats and about the sixth day in the case of adult rats. Routine examinations did not include autopsies, but occasional premature deaths were followed by careful autopsy in search of pneumonia, accidental injury, or cause of death other than uranium poisoning.

Table 6.2 — Mortality in Young Wistar Rats Following Intraperitoneal Injection of Uranyl Nitrate Hexahydrate, $UO_2(NO_3)_2.6H_2O$

Dose		Males		Females	
Mg/kg	Mg U/kg	No. of deaths per no. of rats	Mortality, %	No. of deaths per no. of rats	Mortality, %
4	1.90	15/20	75	19/20	95
5	2.37	13/20*	65	19/20*	95
5	2.37			18/20	90
5	2.37			20/20	100

Approximate LD_{50} = 2 mg U/kg for males; 1 mg U/kg for females

*Litter mates.

(a) Uranyl Nitrate Hexahydrate, UO_2F_2, and UCl_4; Young Rats. The mortality in young Wistar rats following the intraperitoneal injection of uranyl nitrate is presented in Table 6.2. Doses of 4 and 5 mg of the nitrate per kilogram were given to groups of 20 rats of each sex. The percentage mortality was greater for female rats (90 to 100 per cent) than for male rats (65 to 75 per cent). This difference is made more reliable by the fact that litter-mate rats were divided into two of the large groups. Approximate LD_{50}'s were 2 and 1 mg U/kg for males and females, respectively.

Table 6.3—Mortality in Adult Wistar Rats Following Intraperitoneal Injection of Uranyl Nitrate Hexahydrate, $UO_2(NO_3)_2.6H_2O$

Dose		Males		Females	
Mg/kg	Mg U/kg	No. of deaths per no. of rats	Mortality, %	No. of deaths per no. of rats	Mortality, %
5	2.37	4/10	40	8/10	80
5	2.37	3/10	30	7/10	70
5	2.37			18/20	90
5	2.37			19/19	100
5	2.37			19/20*	95
5	2.37			20/20†	100
6	2.84	12/20*	60		
6	2.84	11/20†	55		
8	3.79	12/12	100		

Approximate LD_{50} = 2.5 mg U/kg for males and 1 mg U/kg for females

* Males so marked were litter mates of females so marked.

† Males so marked were litter mates of females so marked.

Adult Rats. The mortality in adult Wistar rats following intraperitoneal injection of uranyl nitrate was determined (Table 6.3). Rats of both sexes were given doses of 5 to 8 mg of uranyl nitrate hexahydrate per kilogram. The mortalities were from 30 to 100 per cent for male rats and 70 to 100 per cent for female rats. This sex difference in mortality is probably real, since brother-sister litter mates were used in several of the large groups. Approximate LD_{50}'s were 2.5 and 1.0 mg U/kg for males and females, respectively. Comparison of Tables 6.2 and 6.3 indicates no age difference in mortality.

Exactly the same technique was used for the determination of mortality in Wistar rats of both sexes following intraperitoneal injections of uranyl fluoride. Two groups of young rats received 2.5 mg of UO_2F_2 per kilogram; mortalities were 70 per cent for the males and 75 per cent for the females (Tables 6.4 and 6.5). Adult male rats received doses of 1.25 to 4.0 mg/kg; the mortalities were from 10 to 95 per cent, indicating an approximate LD_{50} of 2.5 mg U/kg (Table 6.4). Adult female rats that received doses of 1.25 to 2.5 mg/kg had mortalities from 50 to 100 per cent; the approximate LD_{50} was 1.0 mg U/kg (Table 6.5). Hence the toxicity of uranyl fluoride was greater for the females than for the males and greater than the toxicity of uranyl nitrate.

Table 6.4 —Mortality in Male Wistar Rats Following Intraperitoneal Injection of Uranyl Fluoride, UO_2F_2

Dose				
Mg/kg	Mg U/kg	Age	No. of deaths per no. of rats	Mortality, %
2.5	1.95	Young	14/20	70
1.25	0.97	Adult	1/10	10
2.5	1.93	Adult	4/20	20
2.5	1.93	Adult	1/10	10
2.5	1.93	Adult	3/10	30
4.0	3.09	Adult	19/20	95

Approximate LD_{50} = 2.5 mg U/kg (adult rats)

Table 6.5 —Mortality in Female Wistar Rats Following Intraperitoneal Injection of Uranyl Fluoride, UO_2F_2

Dose				
Mg/kg	Mg U/kg	Age	No. of deaths per no. of rats	Mortality, %
2.5	1.93	Young	15/20	75
1.25	0.97	Adult	5/10	50
1.75	1.35	Adult	15/15	100
2.5	1.93	Adult	19/20	95
2.5	1.93	Adult	19/20	95
2.5	1.93	Adult	20/20	100
2.5	1.93	Adult	7/10	70
2.5	1.93	Adult	8/10	80

Approximate LD_{50} = 1.0 mg U/kg (adult rats)

Two groups of young adult rats (one group of male and one group of female rats) received 25.0 mg of uranium tetrachloride per kilogram. The mortality in each group was 90 per cent (Table 6.6).

(b) Comparative Toxicity of Uranyl Nitrate Hexahydrate, UO_2F_2, and UCl_4. Under the conditions of these experiments the nitrate and fluoride were found to be of the same order of toxicity in both young and adult rats. Somewhat larger doses are apparently required to kill the average male than to kill the average female rat. Thus 2 to 2.5 mg U/kg is the approximate LD_{50} for males, whereas 1 mg U/kg is about the average LD_{50} for females. It should be emphasized that the young male and female rats referred to were of the same age and, therefore, of different body weights. It is surprising, in view of the marked difference in mortalities at 24 hr (see preceding section) when rats of different ages were compared, to find little evidence of a dependence of mortality on age for the 2- or 3-week period. Apparently the longer time interval permits the lethal response to be based on some common factor in young and in old rats, perhaps the similarity of kidney injury.

Table 6.6—Mortality in Young Wistar Rats Following Intraperitoneal Injection of Uranium Tetrachloride, UCl_4

Sex	Dose of UCl_4, mg/kg	No. of deaths per no. of rats	Mortality, %
Male	25.0	9/10	90
Female	25.0	9/10	90

In the animals dying in 24 hr, the doses were huge as compared to doses used in the 2- to 3-week studies under discussion. This suggests that some different mechanism of death was involved.

The data for rats given UCl_4 suggest that the dose to kill the average rat was considerably larger than that of nitrate or of fluoride. It is difficult to make more than a rough estimate, but it would seem to take perhaps twice as much of UCl_4.

(c) Summary. 1. Doses of 4 to 5 mg of uranyl nitrate hexahydrate per kilogram produced in young Wistar rats mortalities of about 65 per cent for males and 95 per cent for females.

2. Doses of 5 to 6 mg of uranyl nitrate hexahydrate per kilogram in adult Wistar rats gave mortalities of about 50 per cent in males and about 95 per cent in females.

3. Doses of 2.5 mg of uranyl fluoride per kilogram in male Wistar rats gave mortalities of 20 per cent. In female rats the mortalities were 95 per cent.

4. A dose of 25.0 mg of UCl_4 per kilogram gave a mortality of 90 per cent in adult male and female rats.

2.3 Mortality within 14 to 21 Days, Mice. Albino mice were kept in groups of 10 on shavings in glass jars with wire tops. They received a diet of Purina Rabbit Checkers and drank water from inverted bottles. The C_3H mice were kept under the same conditions in groups of five or less.

(a) Uranyl Nitrate in White and C_3H Mice. Following intraperitoneal injection of uranyl nitrate hexahydrate into white mice in doses varying from 4 to 32 mg/kg, mortalities of 56 to 100 per cent were obtained (Table 6.7). The approximate LD_{50} equaled 6 to 8 mg U/kg.

Doses of 4 to 130 mg of uranyl nitrate hexahydrate per kilogram were given to 135 male C_3H mice (Table 6.8). Mortalities varied from 0 to 100 per cent. The approximate LD_{50} was 25 mg U/kg.

Table 6.7 — Mortality in the White Mouse Following Intraperitoneal Injection of Uranyl Nitrate Hexahydrate

Dose		No. of deaths per no. of mice	Mortality, %
Mg/kg	Mg U/kg		
4.2	1.99	8/9	89
7.7	3.65	5/9	56
11.5	5.45	5/9	56
16.6	7.87	10/10	100
32.0	15.17	9/9	100

Approximate LD_{50} = 6 to 8 mg U/kg

In a total of 135 female C_3H mice given doses of 4 to 150 mg of uranyl nitrate hexahydrate per kilogram, the mortalities varied from 0 to 100 per cent. The approximate LD_{50} was 20 to 24 mg U/kg (Table 6.9). Uranyl nitrate is approximately three or four times as toxic for

Table 6.8 — Mortality in the Male C_3H Mouse Following Intraperitoneal Injection of Uranyl Nitrate Hexahydrate

Dose		No. of deaths per no. of mice	Mortality, %
Mg/kg	Mg U/kg		
3.6	1.71	1/10	10
5.4	2.56	1/10	10
7.8	3.70	0/10	0
12.1	5.74	0/10	0
18.1	8.58	0/5	0
35.7	16.92	1/5	20
50.0	23.70	8/10	80
51.7	24.51	3/10	30
69.0	32.71	10/10	100
77.8	36.83	8/10	80
89.3	42.33	8/10	80
90.0	42.66	9/10	90
107.1	50.77	5/5	100
122.2	57.92	10/10	100
130.0	61.62	7/10	70

Approximate LD_{50} = 25 mg U/kg

the white mouse as for the C_3H mouse. The toxicity of uranyl nitrate for adult Wistar rats was from two to three times that for the white mouse and ten times that for the C_3H mouse.

(b) Summary. 1. Doses of 4 to 32 mg of uranyl nitrate hexahydrate per kilogram gave an approximate LD_{50} of 6 to 8 mg U/kg in white mice.

Table 6.9—Mortality in the Female C_3H Mouse Following Intraperitoneal Injection of Uranyl Nitrate Hexahydrate

Dose		No. of deaths	Mortality,
Mg/kg	Mg U/kg	per no. of mice	%
4.4	2.09	0/10	0
6.3	2.99	0/10	0
10.2	4.83	0/10	0
15.4	7.30	0/10	0
20.3	9.62	1/5	20
47.6	22.56	1/5	20
50.0	23.70	8/10	80
60.0	28.44	9/10	90
83.3	39.48	10/10	100
90.9	43.09	7/10	70
100.0	47.40	7/10	70
125.0	59.25	8/10	80
125.0	59.25	9/10	90
136.4	64.46	4/5	80
150.0	71.10	10/10	100

Approximate LD_{50} = 20 to 24 mg U/kg

2. Doses of 4 to 150 mg of uranyl nitrate hexahydrate per kilogram in male and female C_3H mice gave an approximate LD_{50} of 20 to 25 mg U/kg.

3. MORTALITY FOLLOWING INTRAVENOUS ADMINISTRATION

A preliminary study on rabbits, guinea pigs, rats, and mice was carried out by Orcutt. The program of the study was the same in each species and followed the customary preliminary procedure of placing one animal at each dosage level starting at 0.01 mg/kg and thereafter increasing the dose serially by a factor of 1.67. Various concentrations of uranyl nitrate solutions were used so that the total quantity injected was not less than 0.02 ml nor greater than 2 per cent of the body weight.

The mortalities were recorded daily, and body weights for each animal were obtained daily until death, or until all the survivors of a given species showed consistent weight gains. The rabbits were followed for a maximum of 22 days, guinea pigs for 20 days, rats for 21 days, and mice for 29 days. In Table 6.10, the condition at the end of the observation period is indicated as "normal," "sick," or "dead." The approximate dose that represented the lethal amount is easily apparent from inspection of the table.

Table 6.10—Intravenous Injection of $UO_2(NO_3)_2.6H_2O$

Dose, mg/kg	Rabbits	Guinea pigs	Rats	Mice
0.01	Normal	Normal	Normal	Normal
0.02	Normal	Normal	Normal	Normal
0.03	Normal	Normal	Normal	Normal
0.05	Normal	Normal	Normal	Normal (?)
0.08	Sick	Normal	Normal	Normal
0.13	Normal	Normal	Normal	Normal
0.20	Dead	Normal	Normal	Normal
0.40	Dead	Normal (?)	Normal	Sick
0.60	Dead	Dead	Normal	Normal
1.0	Normal	Dead	Sick	Normal
2.0	Dead	Dead	Dead	Normal
3.0	Dead	Dead		Normal
4.0		Dead	Dead	Sick
6.0	Dead		Dead	
8.0	Dead	Dead	Dead	Normal (?)
13.0	Dead	Dead	Dead	Sick
20.0	Dead	Dead	Dead	Sick
40.0	Dead	Dead	Dead	Dead
60.0		Dead	Dead	Dead
100 and 200		Dead	Dead	Dead

3.1 *Uranyl Nitrate Hexahydrate in Rabbits, Guinea Pigs, Rats, and Mice.* All the rabbits given doses less than 0.1 mg U/kg lived, whereas all but one of the rabbits receiving a higher dose died. Therefore 0.1 mg U/kg may be taken as the approximate lethal dose in rabbits.

All the guinea pigs receiving 0.2 mg U/kg and lesser amounts survived. All those receiving 0.3 mg U/kg and greater amounts died. Therefore 0.3 was the approximate lethal dose for guinea pigs.

Rats that received 0.5 mg U/kg or less survived. Rats that received 1.0 mg U/kg or greater died. Therefore 1.0 mg U/kg was the approximate lethal dose for rats.

Mice receiving 10 mg U/kg or less survived; mice receiving 20 mg U/kg or greater died. Therefore the lethal dose for white mice used in this experiment lay between 10 and 20 mg U/kg.

The values shown in Table 6.10 compare favorably with data on rats and mice reported in the preceding sections and with the data on rabbits from the literature. Rats given uranyl nitrate hexahydrate solutions intraperitoneally required doses of the order of 1 to 2.5 mg U/kg to kill the average rat (see Tables 6.2 and 6.3). The intravenous lethal dose was of the same order of magnitude.

The intraperitoneal dose of uranyl nitrate hexahydrate to kill the average mouse was 6 to 20 mg/kg, depending on the strain (see Tables 6.7, 6.8, and 6.9). Given intravenously, an approximate lethal dose of 10 to 20 mg/kg was obtained. These values are of the same order of magnitude.

Hunter reported values that indicated that about 0.2 mg of uranium per rabbit would be lethal. The value of 0.1 mg U/kg given in Table 6.10 is of the same order of magnitude.

The over-all correspondence of values for the various species obtained by the different investigators was excellent. It is plain that rabbits are more susceptible than guinea pigs, which in turn are more susceptible than rats, and that mice are most resistant. Expressed as approximate numbers, the lethal intravenous dose for rabbits is 0.1, for guinea pigs 0.3, rats 1.0, and mice 10 to 20 mg U/kg. The greater susceptibility of rabbits over guinea pigs and rats has been repeatedly found in inhalation and feeding experiments.

3.2 Summary. The approximate lethal dose of a uranyl nitrate hexahydrate solution administered intravenously in four species of animals is as follows: rabbits, 0.1 mg U/kg; guinea pigs, 0.3 mg U/kg; rats, 1.0 mg U/kg; mice, 10 to 20 mg U/kg.

BIBLIOGRAPHY

1. W. deB. MacNider, J. Exptl. Med., 49: 411 (1929).
2. W. deB. MacNider, Proc. Soc. Exptl. Biol. Med., 32: 791 (1934-35).
3. W. deB. MacNider, J. Pharmacol. Exptl. Therap., 56: 359 (1936).
4. G. L. Donnelly and R. L. Holman, J. Pharmacol. Exptl. Therap., 75: 11 (1942).
5. G. E. Gustafson, S. Koletsky, and A. H. Free, Arch. Internal Med., 74: 416 (1944).
6. W. C. Hunter, Ann. Internal Med., 1: 747 (1928).
7. C. L. Bliss, Ann. Applied Biol., 22: 134 (1935).

Chapter 7

STUDIES OF THE TOXICITY OF VARIOUS URANIUM COMPOUNDS WHEN FED TO EXPERIMENTAL ANIMALS

By Elliott A. Maynard and Harold C. Hodge*

1. INTRODUCTION

It is generally recognized that the most important and at the same time the most insidious industrial exposures to toxic agents are inhalation exposures. The bulk of experimental work on uranium toxicity has involved exposures of animals to dust-laden air. Such exposures are not simple, uncomplicated lung exposures; the animals also receive toxic material through the gastrointestinal tract and possibly through the skin.

The gastrointestinal intake may arise from at least two important sources:

1. Large particles of dust may settle in the nose, pharynx, or in the upper ciliated portions of the bronchi. For soluble dusts, solution and absorption may occur in greater or less degree. For insoluble dusts the particles are mostly passed into the gastrointestinal tract from which they are absorbed to a variable and unknown extent, and the balance is excreted.
2. The animals may attempt to lick clean the fur on which toxic dusts have settled. For certain dusts this may constitute a high order of exposure. Toxic dusts that have settled to the floor may also be picked up on the pads of the paws, which are cleaned by teeth and tongue; this provides an additional source of ingested uranium compounds.

If it could be demonstrated that feeding relatively large amounts of a given material produced no mortality and only slight evidences of injury by other criteria, then it seemed reasonable that in animals exposed to this material via the lung the contribution of ingested substance to the over-all toxicity would be discounted. On the other hand, if extremely minute amounts in the diets of animals gave rise to severe poisoning, every effort should be made to reduce the oral exposure to a minimum. Thus it became important to assess carefully the oral toxicity of each of the uranium compounds for which the inhalation toxicity was to be studied.

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*Work done under the direction of Elliott A. Maynard and Harold C. Hodge with the assistance of William Downs and Raymond Kesel.

2. THIRTY-DAY FEEDING EXPERIMENTS, RATS

Nine uranium compounds were submitted to the project laboratory in June 1943, with a request for information concerning the toxicities as soon as possible. For most of these compounds no toxicological data were available. When work was initiated on the project, it was apparent that approximate relative toxicities could be obtained quickly in a satisfactory form by feeding groups of rats various levels of the more important compounds. It was expedient to start by feeding large amounts over a short period.

The plan frequently followed was to feed four groups of 15 to 25 rats each at the following levels in the diet: 0, 0.5, 2, and 20 per cent. From the graded responses to the wide differences in intake that these percentages represent, the compounds could be promptly classified as "nontoxic," "moderately toxic," or "severely toxic." The rats were observed for their clinical conditions; the body weights were recorded weekly. In most of the experiments a few periodic urine samples were tested for sugar and albumin, and a few blood counts were made. At the end of a month, more or less, the rats were sacrificed, and autopsies were performed, organ weights were determined, and tissue samples were taken for histological examination.

2.1 Approximate Toxicities. Following this general plan, pilot experiments were run on a number of compounds, which are divided in

Table 7.1 — Approximate Toxicities of Uranium Compounds

	Compounds
Nontoxic*	UO_2
	U_3O_8
	UF_4
Toxic†	UO_3
	UO_2Ac_2
	UO_4
	UCl_4
	UO_2F_2
	$UO_2(NO_3)_2.6H_2O$

*Little effect was observed when the diet contained 20 per cent.
†Growth diminished when the diet contained 1 per cent or less.

Table 7.2—Numbers of Male and Female Rats Fed Various Uranium Compounds for 30 Days*

Percentage of compound in diet	UO_2F_2	$UO_2(NO_3)_2$ $.6H_2O$	UCl_4	UF_4	UO_2	UO_4	U_3O_8	UO_3	UO_2Ac_2
20.0 (M)	10	15		38	25		7	15	15
20.0 (F)	10		15	37	20	15	8		
10.0 (M)		8							
10.0 (F)		7							
5.0 (M)	15	6							
5.0 (F)	15	9							
3.0 (M)			15						
3.0 (F)		15	10						
2.0 (M)	10	323		38	25	15	7	15	15
2.0 (F)	10	308	15	37	20	15	8		
1.5 (M)			15						
1.5 (F)			10						
1.0 (M)	15		30			26			
1.0 (F)	15	15	25			4			
0.75 (M)	15								
0.75 (F)	15								
0.5 (M)	25	198	15	38	25	15	7	15	15
0.5 (F)	25	183	30	37	20	15	8		
0.25 (M)	70					11			
0.25 (F)	70					4			
0.2 (M)			15						
0.2 (F)			15						
0.1 (M)	15	153				15			
0.1 (F)	15	153							
0.05 (M)	40								
0.05 (F)	40								
0.01 (M)	25								
0.01 (F)	25								
			Totals						
Totals (M)	240	703	90	114	75	82	21	45	45
Totals (F)	240	690	120	111	60	53	24		

* 958 (M) and 958 (F) used as controls, with no uranium compound.
(M) signifies male; (F) female.

Table 7.1 according to their approximate toxicities as determined. Thus, from the 1-month studies, three compounds were shown to be relatively nontoxic when taken orally by rats; six of the uranium compounds were found to be toxic.

The nontoxic compounds fed at levels of 0.5, 2, and 20 per cent of the diets produced no depression of growth and no mortalities, except one female rat fed 20 per cent UF_4. Another important exception to this statement must be made: 20 per cent UF_4 in the diets produced a marked loss of growth, which amounted to 15 to 49 g in male rats and 5 to 17 g in the female rats.

2.2 Dietary Levels. Feeding the toxic compounds at levels of 20 per cent of the diet frequently killed all the animals in a few days. In fact, in the case of the more toxic compounds, 2 per cent killed most of the rats within a week. Consequently for a number of compounds additional 1-month pilot experiments were set up in which various lower levels of dietary intake were used (Table 7.2). The magnitude of these tests may be appreciated by considering that 1,415 male rats and 1,298 female rats were fed experimental diets at the same time that 958 male and 958 female rats were fed stock rations as specific controls. This total group included 4,629 rats.

2.3 Growth Curves. The growth curves of some of the groups of male and female rats fed various amounts of uranyl nitrate are shown in Figs. 7.1 and 7.2. In male rats, 2.0 per cent uranyl nitrate hexahydrate produced a marked loss of growth that amounted to 40 or 50 g

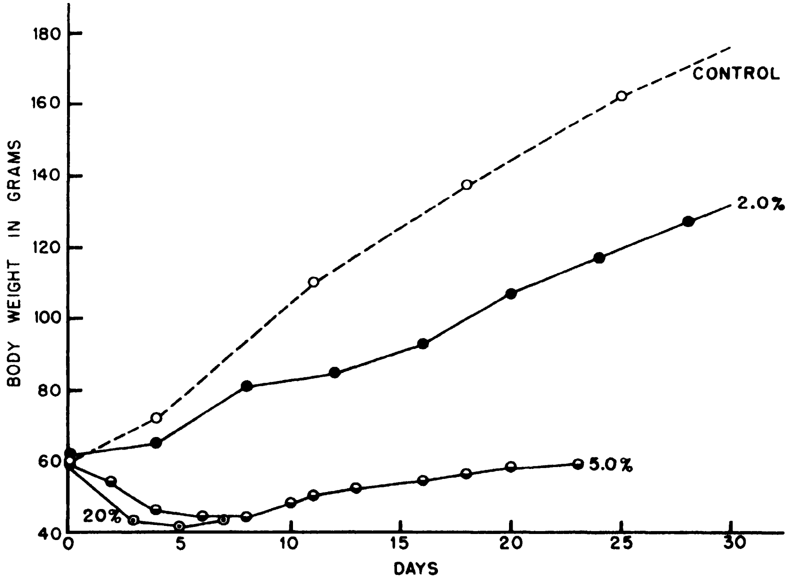

Fig. 7.1—Growth curves for male rats fed various levels of uranyl nitrate hexahydrate in the diet for about 1 month.

at the end of 1 month. Rats that received 5 per cent uranyl nitrate hexahydrate suffered a depression of body weight; many rats died in the first week; the survivors slowly gained and reached almost exactly their original weight. All the rats, however, on this level (5 per cent) were dead at the end of a little more than 3 weeks. When male rats were fed 20 per cent uranyl nitrate hexahydrate in the diets, they were all dead at the end of 7 days.

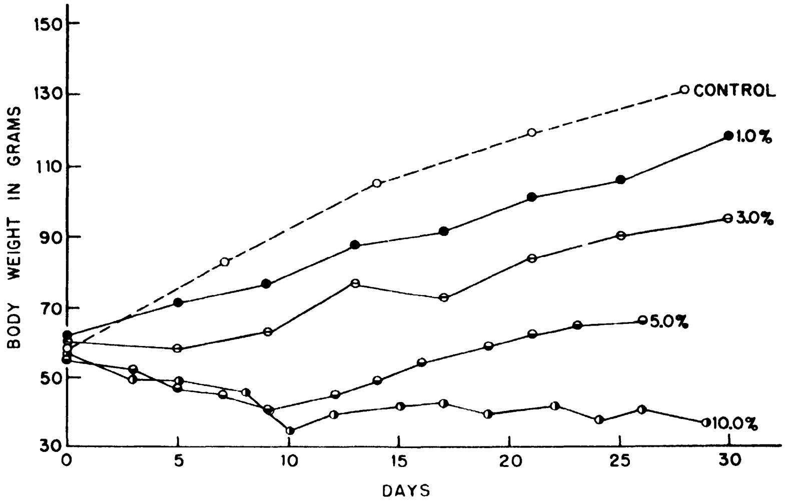

Fig. 7.2—Growth curves for female rats fed various levels of uranyl nitrate hexahydrate in the diet for about 1 month.

Figure 7.2 shows the growth curves of female rats fed 0, 1, 3, 5, and 10 per cent uranyl nitrate hexahydrate. One per cent produced a depression of about 10 g at the end of 1 month. Three per cent produced a depression of 30 g with a concomitant 30 per cent mortality. Five per cent produced a depression of 50 g and higher mortality (67 per cent). Ten per cent produced a 90-g weight depression; the rats never regained their original weight, and mortality was 86 per cent.

These two sets of growth curves (with corresponding mortality figures) illustrate the kinds of records that were obtained in the short-term feeding experiments. It would be impossible to reproduce here even average data on the many tests. However, from Table 7.2 it may be seen that a large number of such feeding experiments have been carried out.

2.4 Lethal Level in Diets. One of the ways in which the toxicities of these compounds may be compared is by the mortalities observed during 30-day feeding experiments. Table 7.3 gives for several of the compounds the dosages producing (1) 100 per cent mortality, (2) 50 per cent mortality, (3) no mortality in male and female rats. From this table, it may be seen that 2 to 10 per cent of certain compounds gave 100 per cent mortality. It might be added that 1 to 5 per cent of these compounds failed to be uniformly lethal. These compounds were considered to be the more toxic compounds. Note that UO_2F_2, UCl_4, and UO_4 given at 1 to 2½ per cent of the diet will produce a 50 per cent mortality, whereas 4 per cent uranyl nitrate hexahydrate in the diet is required for the same effect. The approximate LL_{50} (lethal level in the diets to kill half of the rats) may be estimated by plotting the percentage mortality against the percentage of compound in the diet (Fig. 7.3). In the case of uranyl nitrate, typical S curves were obtained for male and for female rats with LL_{50}'s of the order of 4 per cent uranyl nitrate hexahydrate in the diet. This is the fashion in which the 50 per cent mortality doses given in Table 7.3 were

Table 7.3—Mortality in 30-day Feeding Tests*

Compound	Sex	Dosage producing 100% mortality, %	Approximate dosage producing 50% mortality, %	Maximum dosage failing to produce any mortality, %
UO_2F_2	Male	5.0	1	0.5
	Female		1	0.5
UCl_4	Male	3.0	1.5	0.5
	Female	20.0	2.5	1.0
UO_4	Male	2.0	1.5	0.25
	Female	20.0	2	1.0
$UO_2(NO_3)_2.6H_2O$	Male	10.0	4	0.5
	Female		4	0.5
UO_3	Male	2.0		0.5
UO_2Ac_2	Male	20.0		0.5
UO_2	Male and female			20.0
U_3O_8	Male and female			20.0
UF_4	Male and female			20.0†

*Numbers in the body of the table are percentages in the diets.
†One female died.

estimated. It should be pointed out that the amounts of these compounds necessary to produce 100 per cent mortality in rats were fairly large. On a weight-for-weight basis, 4 per cent of the diet of a rat would correspond to an intake of 4 lb/week in a 70-kg individual.

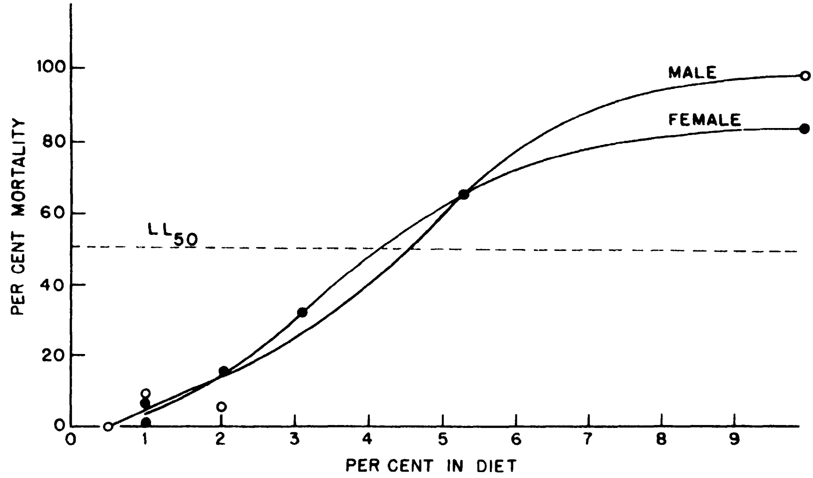

Fig. 7.3 —Mortality in 30-day feeding tests with uranyl nitrate hexahydrate in rats.

2.5 Amounts Producing a Decreased Growth. An interesting characterization of the data was obtained by plotting the logarithms of the percentages in the diets vs. the logarithm of the corresponding weight depressions. By "weight depression" is meant the difference between the average weight of control rats and the average weight of the experimental rats. In each case the weights were those at termination of the experiments. An illustration of this relationship is given in Fig. 7.4, where the data for uranyl nitrate are given for male and for female rats. Similar curves were obtained for the other compounds. Several points were derived from this manipulation of the data.

1. There was a marked consistency of response. Some of the groups, e.g., those fed uranyl nitrate (Fig. 7.4), showed almost linear relationships. Since these groups of rats were fed at different times of the year and since they were not all litter mates, such consistency was taken as evidence of a marked degree of reliability of the measured toxic response.

2. There were noteworthy differences between the slopes of the lines for the different compounds. Thus, UO_2F_2 appeared to be the most toxic; the curves were far to the left in the chart and rose steeply. The compound UF_4 appeared to be the least toxic; the curves

were far to the right and had a gentle upward slope. The curves for the other compounds were intermediate. One way in which a quantitative comparison can be made is by interpolating from the lines in these graphs the dietary percentages corresponding to a 50-g weight depression.

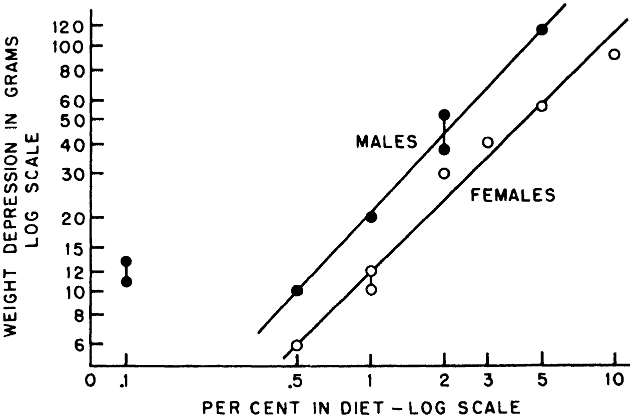

Fig. 7.4—Weight depression in rats fed various levels of uranyl nitrate hexahydrate for 30 days.

Compound	Percentage in diet giving 50-g weight depression	
	In males	In females
UO_2F_2	0.6	0.85
UCl_4	0.6–0.9	0.8–2.2
UO_4	0.8	2.2
$UO_2(NO_3)_2.6H_2O$	2.4	4.2
UO_3	3.5	
UF_4	20	

3. The female rats exhibited a lesser response as measured by weight depression than did the male rats. This difference certainly cannot be entirely attributed to the fact that the female weighs less than the male. At the end of 30 days the average control male rat weighed 180 to 200 g, whereas the average control female weighed 130 to 145 g. The percentage failure to grow was greater in the male than in the female rat fed uranyl nitrate. For example, male weanlings

after 30 days on a diet containing 2 per cent of uranyl nitrate hexahydrate weighed 154 g on the average as compared with 212 g for the control group; this is a 58-g difference, and represents a 27 per cent weight depression (Fig. 7.8). The comparable group of female rats weighed 123 g as compared with 152 g for the control group; this is a 29-g difference and represents a 19 per cent weight depression (Fig. 7.9).

In Fig. 7.5, block graphs illustrate the average body weight of female rats fed 2 per cent of various uranium compounds in the diets for 30 days. In each case the control rat's weight is taken as zero. At the left of the figure it is evident that the rats fed UO_2, U_3O_8, and UF_4 weighed somewhat more, but probably not significantly more, than the control rats. In the center of the diagram it may be seen that rats fed UO_3, uranyl nitrate, UCl_4, and UO_4 lost 35 to 50 g as compared with the control. (Note that the data for UO_3 are on male rats.) At the right of the figure the unique toxicity of UO_2F_2 is shown by the weight depression of nearly 80 g.

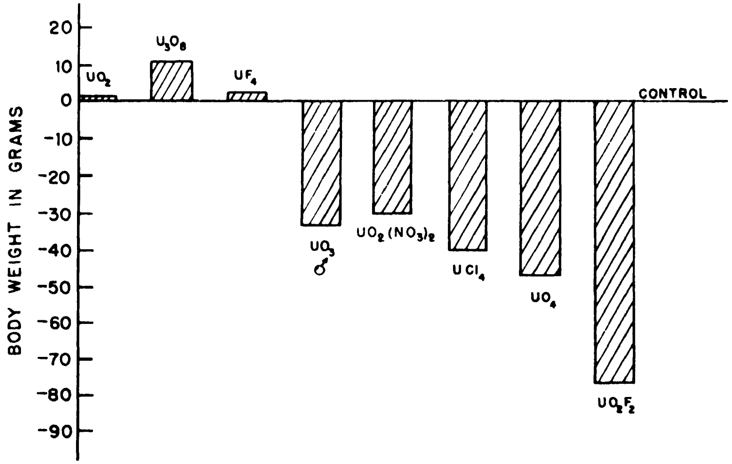

Fig. 7.5—Average body-weight changes in rats fed 2 per cent of various uranium compounds in the diet for 30 days. All rats were females except the group fed UO_3.

One of the ways of comparing these compounds is to note the smallest amounts in the diets that produced a detectable poisoning as shown by a decrease in growth rate. These compounds may thus be grouped on the basis of the least amounts that gave loss of growth as shown in Table 7.4.

Table 7.4—Smallest Amounts of Uranium Compounds Producing Growth Depression in Rats

Amount in diet, %	Compounds	Effect on growth
0.1-0.25	UO_4 UO_2F_2	Some interference
0.5-1	$UO_2(NO_3)_2.6H_2O$ UO_3 UCl_4 UO_2Ac_2	Some interference
20	UO_2 U_3O_8	No interference
20	UF_4	Some interference

2.6 Age Differences, Weanling Rats vs. Mature Rats. Mature rats were much more susceptible to uranium poisoning than were weanlings. One of the most impressive comparisons of the differences in toxicity for weanling rats and for adult rats (6 months old) may be obtained by observing the mortalities following the ingestion for 1 month of a diet containing 2 per cent uranyl nitrate hexahydrate (Fig. 7.6). There were at both ages practically no deaths in the first week; almost all the deaths occurred during the second week. Only a few of the 125 young male rats died (12 per cent); a somewhat larger percentage (21 per cent) of the 125 young female rats died. These values are in sharp contrast with the marked mortality of mature rats in which 76 per cent of the 50 males and 68 per cent of the 50 females died.

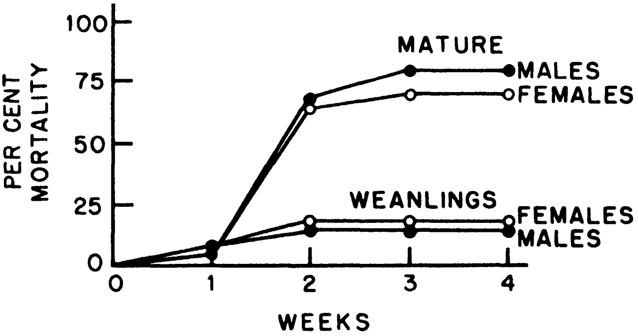

Fig. 7.6—Mortalities in weanling and mature rats fed 2 per cent uranyl nitrate hexahydrate.

The susceptibility of the mature rats was much greater than that of the weanling rats. This difference in poisonous effects is clearly shown in Fig. 7.7, in which body weights are plotted as percentages of the respective initial weights for the 4-week feeding interval. In the case of the mature rats shown at the bottom of the graph, the body weights decreased in almost linear fashion for the first 2 weeks, after which there were slow weight gains. However, at the end of the experiment the starting weights had not been regained. In contrast, although the weanling rats lost weight during the first week at almost the same rate as the mature rats, thereafter the weanlings gained weight in a rapid fashion so that at the end of the month the female weights averaged 147 per cent and the male weights averaged 176 per cent of the initial body weights. These curves, together with the mortality differences shown in Fig. 7.6, present clearly the markedly higher susceptibility to uranium poisoning of the adult rats as compared with the weanling rats.

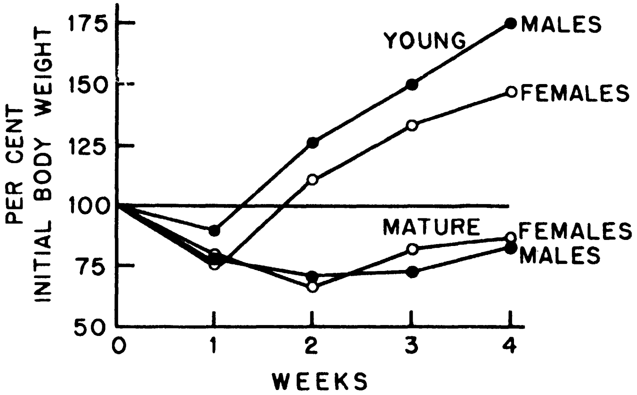

Fig. 7.7 —Body-weight changes of weanling and mature rats fed 2 per cent uranyl nitrate hexahydrate.

2.7 Hematology. In general, the hematological studies showed no significant changes as a result of any of the feeding experiments. The only exception to this statement occurred in the case of the mature rats fed 2 per cent uranyl nitrate hexahydrate, in which small decreases in red count, hemoglobin, and reticulocyte percentages were observed. The significance of this observation is not known.

2.8 Pathology. At the time of sacrifice, gross autopsies were performed, and samples of a number of tissues were immediately placed in fixative solutions for ultimate histological examination. In most of the experiments the following tissues of each rat were examined: lung, heart, stomach, small intestine, large intestine, liver, spleen, and

Table 7.5 — Frequency and Degree of Renal Change in Representative Groups of Weanling Rats Fed Various Uranium Compounds for 30 Days

Compound	Dietary level, %	No. of rats	Frequency[*]	Degree[†]
$UO_2(NO_3)_2 \cdot 6H_2O$	0.1	3		N
	0.5	3	2/3	1
	2.0	3	3/3	3
	0.1	6 (mature)[‡]	1/6	<1
	0.5	6 (mature)[‡]	6/6	1
	2.0	6 (mature)[‡]	6/6	2 – 3
UO_2	0.5	15		N
	2.0	15		N
	20.0	15		N
UF_4	0.5	30		N
	2.0	30		N
	20.0	30	5/30	<1
UO_4	0.25	15	13/15	<1
	0.50	15	13/15	1 – 2
	0.2	8	8/8	2 – 3
UO_3	0.5	15		N[§]
	2.0	15		N[§]
UO_2Ac_2	0.5	15		N
	2.0	13	10/13	2
UCl_4	0.2	30		N
	0.5	45	9/45	<1
	1.0	50	41/50	1 – 2
	1.5	17	17/17	3
	3.0	3	3/3	4
U_3O_8	0.5	15		N
	2.0	15		N
UO_2F_2	0.05	30	1/30	1
	0.1	29	1/29	1
	0.25	60	1/60	1
	0.5	20	5/20	1
	1.0	3	3/3	2
	2.0	9	9/9	2

[*] The numerator of the frequency fraction represents the number of rats with renal change in that group.

[†] N = all normal; <1 = damage just detectable; 1 = mild; 2 = moderate; 3 = moderately marked; 4 = marked.

[‡] These rats were 6 months old when placed on the diets. All other rats listed in this table were weanlings (about 4 weeks old).

[§] On diet 60 days.

kidney. In a few cases, additional studies were made of bone; the femur was the usual tissue sampled. Gonads were taken from a number of rats.

The kidney showed evidence of histological injury. The only other pathological changes found were occasional abnormalities in the lung, e.g., pneumonia, and occasional examples of parasitism of the intestine, or of the liver. No clear-cut correlation of dosage of uranium compound and histological damage was found except in the kidney.

The changes in the kidney were of the type described in Chap. 4. In general, the animals dying within the first week or two on the diet showed evidence of active epithelial damage, and those surviving showed a predominance of regenerative processes at the end of the 30-day period. The extent of the injury and frequency of the occurrence of change varied with the dosage level (see Table 7.5).

2.9 Summary. 1. As a result of 30-day feeding tests with rats, UO_2, U_3O_8, and UF_4 were found to be nontoxic; UO_3, UO_2Ac_2, UO_4, UCl_4, UO_2F_2, and $UO_2(NO_3)_2.6H_2O$ were found to be toxic.

2. Diets with up to 20 per cent of any nontoxic compound caused no mortality and very little interference with growth; 2 to 10 per cent of any toxic compound in the diet caused 100 per cent mortality, and 0.1 to 1 per cent of these compounds in the diet caused growth depression.

3. Mature rats were more susceptible to poisoning by uranyl nitrate in the diet than were weanling rats.

4. The kidneys were the only organs that showed histological damage as a result of uranium poisoning under these conditions.

5. No significant hematological changes were found after feeding uranium compounds for 30 days.

3. FOOD CONSUMPTION BY URANIUM-POISONED RATS

In order to follow the effects of the ingestion of toxic quantities of a uranium compound, groups of young weanling rats (25 of each sex, litter mated) and similar groups of mature rats at the age of 6 months were placed on diets containing 0 and 2 per cent uranyl nitrate hexahydrate, respectively. The experiment was set up to record the food consumption; each rat was fed in a single cage, and a record was kept of the amount of food consumed each day. The records of growth and food consumption are presented for the young and for the old rats, by sexes, in Figs. 7.8 to 7.11.

3.1 Young Male Rats. In a preliminary period of a week the animals became accustomed to the cages, and the daily food intake was measured. On the 9th day the experimental rats received a ration containing 2 per cent uranyl nitrate hexahydrate (Fig. 7.8). The food consumption was almost as large as usual; on that day 13 g of diet was ingested, whereas during the preceding week 11 to 15 g had been consumed daily. The average body weight after the first 24 hr on the diet represented a peak in growth, a value that was not regained until 13 days later. In the succeeding 6 days the average food intake fell off in a nearly linear fashion to about one-third of the normal intake. This anorexia was continued until the 15th day of the experiment, when the food intake began a steady increase toward preexposure amounts.

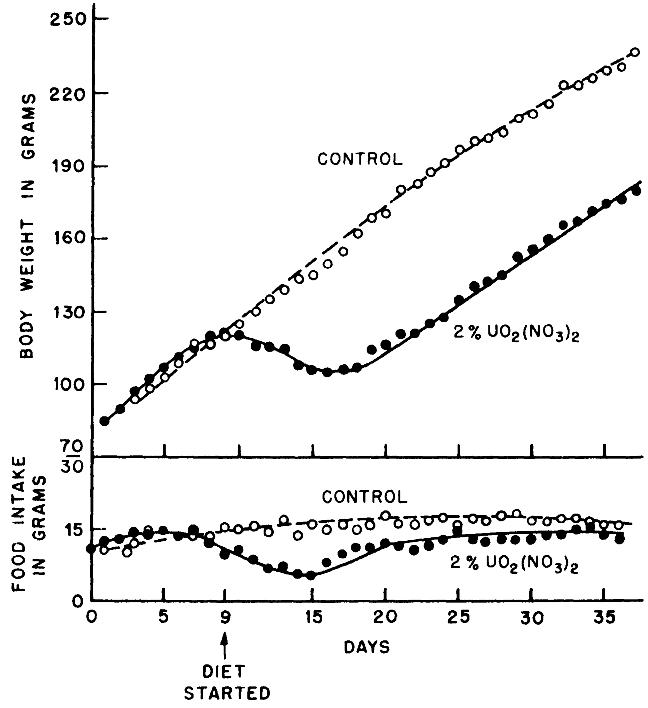

Fig. 7.8—Growth curves (above) and food-consumption curves (below) for weanling male rats fed 2 per cent uranyl nitrate hexahydrate in the diet.

After a lag of 2 or 3 days the body weight started to increase. The food intake continued to increase in an irregular fashion for about 10 days, at which time it had reached the average normal intake for rats of this age, and during the last 10 days of the experiment ranged from 13 to 15 g/day. If the body-weight curve is shifted by 9 days so that the body weight on the 18th day coincides with the body weight of the control rats on the 6th day, the growth curve of the experimental rats for the rest of the experiment can be nearly superimposed on the growth curve for the control rats. For example, at 30 days the experimental rats weighed about 4 g less than the controls had at 18 days. Of course, comparing the over-all growth during the month period, there is a marked growth reduction amounting to 50 g or more by the 35th day, as has been repeatedly observed.

3.2 Young Female Rats. As before, the rats were followed for about a week before the nitrate-containing diet was given. The average female rat ate 12 g on the first day; this compares favorably with the 10 to 15 g that each had eaten during the preceding week (Fig. 7.9).

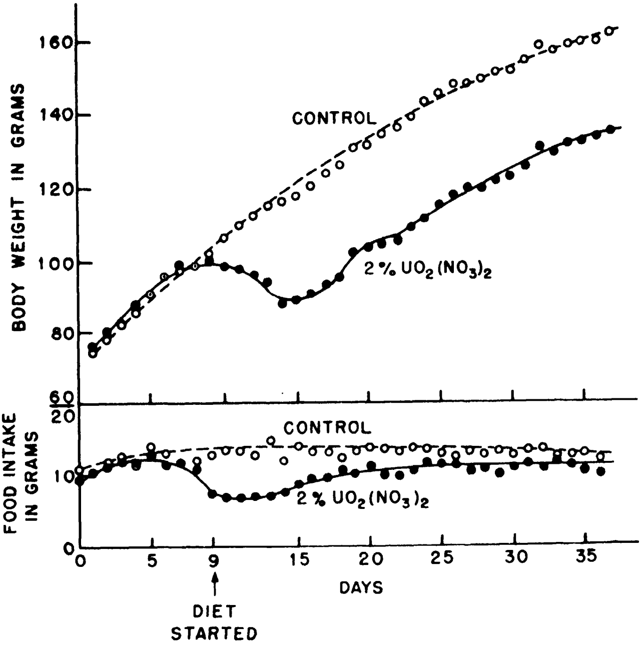

Fig. 7.9—Growth curves (above) and food-consumption curves (below) for weanling female rats fed 2 per cent uranyl nitrate hexahydrate in the diet.

On the next day the dietary intake fell off sharply and continued to diminish to about half the normal intake when the rats had been 5 days on the experimental diet. Body weights had exactly equaled those of the control rats until the day after receiving the nitrate; thereafter the weights decreased in an irregular fashion for 6 days. On this day the food intake began to increase and 6 days later was practically normal (12 g). The weight-increase curve follows closely that of the control if the experimental rats as of the 16th day are compared with the control rats as of the 4th day. The over-all growth pictures are as have been described before; at the end of the month the experimental animals weighed about 30 g less than the litter-mate controls.

3.3 Mature Male Rats. The average intake of diet by these 300-g male rats was about 15 g/day (Fig. 7.10). On the first day of feeding 2 per cent uranyl nitrate hexahydrate, 14 g was eaten on the average.

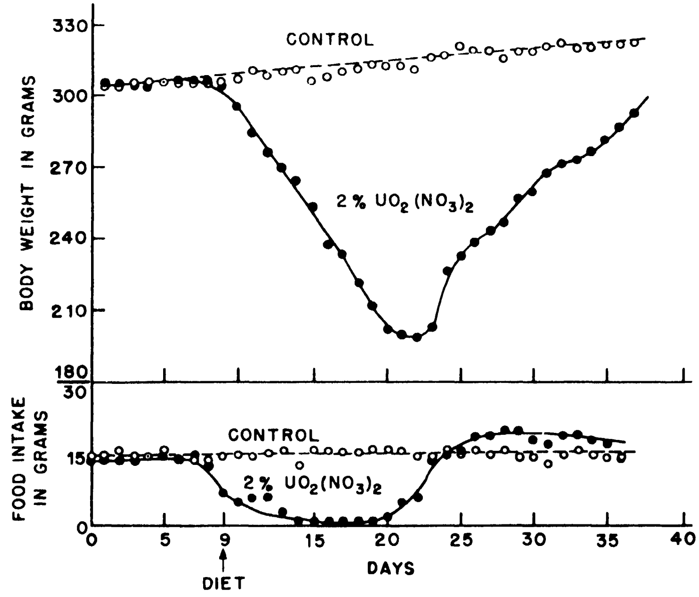

Fig. 7.10—Growth curves (above) and food-consumption curves (below) for mature male rats fed 2 per cent uranyl nitrate hexahydrate in the diet.

The food intake diminished by half on the succeeding day and by the 6th day was down to practically nothing. Only about 1 g of diet was consumed per rat on that day. It is interesting to compare this refusal of food with the anorexia of young rats. The latter never dropped beneath an average intake of 5 g/day. The extra food refused on the part of the mature rats, i.e., the differences between 5 g/day and the amounts eaten, is discussed below. This very low dietary intake was maintained for 6 or 7 days before an increase in food intake was seen. During the 2 weeks following the first day on the diet, the body weight decreased in a nearly linear fashion. This decrease amounted to almost 100 g, or about 30 per cent of the animal's weight. The resumption of feeding preceded by 3 or 4 days an increase in body weight.

However, once established, the trend of recovery of body weight was nearly as rapid and as regular as the loss in weight had been.

A peculiarity of the older animals is clearly shown in the overcompensation and overeating during the recovery period. Thus by the 24th day the average intake had reached the preexposure level of 15 g/day, and thereafter for 12 days the rats consistently overate up to 5 g daily more than their customary intake. It is an interesting fact that the total amount of food overeaten, i.e., in excess of the average intake for a rat this size, was almost precisely equal to the extra amount of food refused during the second to tenth days on the diet containing uranyl nitrate (the period during which the food intake was less than 5 g/day). It should be noted that the rats failed to regain their starting weight at the end of the experiment.

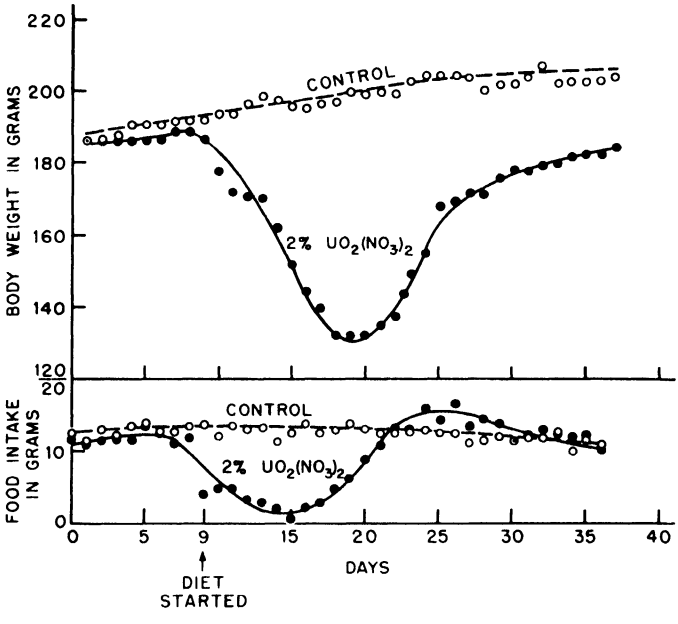

Fig. 7.11—Growth curves (above) and food-consumption curves (below) for mature female rats fed 2 per cent uranyl nitrate hexahydrate in the diet.

3.4 Mature Female Rats. The average female rat, body weight about 180 g, ingested 12 to 14 g of food daily (Fig. 7.11). These rats ate about 12 g each on the first day of the experimental diet. The succeeding day saw the dietary intake fall to about one-third this value, and the intake decreased during the succeeding week to a minimum of about 1 g/rat on the average. On the 15th day the food intake began to increase. Thereafter, for the next 10 days, it showed a remarkably steady increase to something above the normal dietary intake. For the next period (of about 8 days) the rats ingested several grams daily more than the average intake. Again, the excess eaten was roughly equivalent numerically to the deficit in intake on the days when less than 5 g was ingested per rat. The body weights, which had decreased sharply from 185 to 132 g, began to increase on the 20th day, 3 or 4 days after the food intake began to increase. The final body weight was nearly equal to the original weight.

3.5 Discussion. There are several interesting points to be considered in this experiment. The most important is the demonstration that the weight loss and the refusal of diet follows 1 day of ingesting a nearly normal amount of the experimental diet containing the toxic material. Thus the animal has received a large dose of uranyl nitrate, and, as the characteristic cycle of kidney injury develops and the animal becomes sicker, the food intake decreases to a minimum on the 5th to 12th days. This period of minimum food intake coincides precisely with the period of maximum mortality observed in many of the studies, especially the studies of toxicity following inhalation of various compounds. It is not known just how important the extra amount was that the animals ingested on the 2d, 3d, and 4th days. It is probable that the large dose on the first day was sufficient to account for most of the changes noted in the following paragraphs.

One of the interesting aspects was the nearly precise paralleling of weight and dietary changes of the weanlings as compared with the mature rats. The times of weight loss, diminished appetite, weight gain, and increased appetite were in practically identical sequence for both young and old rats.

It was not unexpected that the food consumption would change prior to the change in body weight. This was especially noticeable in the beginning of the recovery period when the animals' appetites picked up 1, or 2, or 3 days ahead of the first increases in average body weights. The curves for body weight and food consumption, as was expected, were closely parallel in trend.

Some comment is required on the growth curves of the weanling rats during the recovery phase. Two points might be mentioned. First, it has been demonstrated and will be discussed in Sec. 4.2 in some detail that rats maintained on a diet of 2 per cent uranyl nitrate hexahydrate for a period of 7 months will thereafter, when placed on stock ration, practically regain their delayed growth impetus, and in an additional 5 months will weigh almost the same as control animals reared for a 1-year period on the stock diet. Thus the failure for the growth curves to be superimposed (Figs. 7.8 and 7.9) is evidence of delayed growth, but not necessarily of permanent loss of growth ability. That is, the animals were not stunted, just retarded. The other point is that what appear to be trends for cyclic toxic effects to show themselves have been repeatedly seen. This cycle of loss of body weight, although not so clearly marked as to be indisputable, has several times been suspected. The span of the cycle seems to be about 1 month. Further work is needed on this point.

3.6 Summary. 1. In both weanling and mature rats, body-weight depression and loss of appetite promptly appeared on the first day following the ingestion of 2.0 per cent uranyl nitrate hexahydrate in the diet.

2. Intake of the diet containing 2.0 per cent uranyl nitrate hexahydrate reached minimal values on the 5th to 12th days, accompanied by the expected mortalities.

3. After recovery from the initial effects of the uranium poisoning, the surviving mature rats overate an amount sufficient to compensate for the earlier period of fasting.

4. ONE-YEAR FEEDING EXPERIMENTS, RATS

At a conference held by the Medical Section in October 1943, a division of projected feeding experiments was made between the Rochester laboratory and Tannenbaum's laboratory at Michael Reese Hospital, Chicago. According to the program set down at that time, chronic feeding experiments on rats would be conducted in Rochester on the following four compounds only: UO_2F_2, uranyl nitrate, UF_4, and UO_2. In this section the data will be presented on the body weights and mortalities of rats fed diets containing various levels of these four compounds over a period of 12 months. In addition, a study of the effects of uranyl nitrate in the diet of a breeding colony of rats will be appended.

4.1 Uranyl Fluoride, Growth Effects. Selected average body weights of the groups of male and female rats fed various percentages of UO_2F_2 in their diets are given in Tables 7.6 and 7.7. The tables contain a statement of the percentage of UO_2F_2 in the diet, the number of rats in the experiment, and average body weights comparable to the control group in each experiment initially and after 1, 2, 3, 4, 5, 6, 8, and 12 months. The experimental groups that are to be compared with the respective controls are indicated by integers placed before the statement of percentage in diet. Thus, for the male rats, Experiment 1 had a group of control rats and groups fed 0.25 and 0.50 per cent UO_2F_2 in the diets. There were 15 rats in each experiment. The initial weight of the control group was 123 g; that of the experimental

Table 7.6—Average Difference in Body Weight of Groups of Male Rats Fed Uranyl Fluoride Compared with the Average Weight of the Control Groups

Exptl. group*	Amount in diet, %	No. of rats per expt.	0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	8 mo.	12 mo.	16 mo.	20 mo.	Comments on growth
			Body wt., g											
1	None	15	123	236	306	318	338	347	356	371	372	390	397	Normal
2	None	25	82	201	252	290	313	337	354	384	393	396		Normal
3	None	25	80	193	255	272	289	315	321	341	369	375	379	Normal
			Weight difference from controls, g											
3	0.01	25	−1	−2	+1	+3	+7	+3	+7	+2	+1	+6	+10	Same as controls
2	0.05	25	−2	0	+3	−1	0	−1	−2	−10	+3	−10		Same as controls
3	0.05	25	−1	+1	+5	+11	+13	+10	+12	+13	+9	−5	+1	Consistently outweighed controls
2	0.1	25	−2	−4	0	−6	−6	−8	−8	−20	−11	−21		Consistently weighed less than controls
2	0.15	25	−2	−5	−5	−13	−16	−18	−16	−41	−33	−23		Marked depression of growth
1	0.25	15	−10	−24	−45	−37	−41	−39	−41	−38	−32	−43	−39	Consistently weighed less than controls
3	0.25	25	+3	−14	−21	−24	−19	−25	−20	−30	−27	−32	−35	Consistently weighed less than controls
1	0.50	15	−33	−61	−117	−108	−112	−100	−118	−110	−112	−108	−85	Marked depression of growth

*To identify the individual experiments, the control and experimental groups are given the same integer.

Table 7.7—Average Difference in Body Weight of Groups of Female Rats Fed Uranyl Fluoride Compared with the Average Weight of the Control Groups

Exptl. group*	Amount in diet, %	No. of rats per expt.	0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	8 mo.	12 mo.	16 mo.	20 mo.	Comments on growth
								Body wt., g						
1	None	15	111	163	191	202	211	213	217	228	239	258	275	Normal
2	None	25	97	152	180	191	196	207	213	220	236	243	262	Normal
								Weight difference from controls, g						
2	0.01	25	-5	-3	-3	-1	+1	+2	+1	0	+4	+16	-7	Same as controls
2	0.05	25	-3	-4	-1	-3	-2	-1	0	+2	-1	+3	-22	Same as controls
1	0.25	15	-6	-7	-13	-11	-15	-14	-12	-15	-15	-25	-35	Consistently weighed less than controls
2	0.25	25	-3	-8	-12	-13	-13	-15	-15	-14	-21	-27	-42	Consistently weighed less than controls
1	0.50	15	-22	-23	-44	-37	-43	-35	-35	-43	-43	-53	-82	Marked depression of growth

*To identify the individual experiments, the control and experimental groups are given the same integer.

groups 113 and 90 g, respectively. At the end of the first month the control group had an average weight of 236 g; the experimental groups had 212 and 175 g, respectively. In a similar fashion the values for the other groups are recorded. Thus for the second experiment there were groups of control rats and rats fed 0.05, 0.1, and 0.15 per cent UO_2F_2 in the diet, respectively. The average weight of the control group at the start of the experiment was 82 g; the experimental groups averaged 80 g each. All the growth tables are constructed in this fashion and the amount of detail given here is to ensure the easy interpretation of the tables.

From the 30-day feeding tests it had been established that rats fed 20, 5, 2, and 1 per cent UO_2F_2 in the diets suffered such severe mortality that feeding experiments of longer duration at these levels were impossible. Just what the maximum dietary content of UO_2F_2 could be without extensive mortality was unknown when the chronic feeding experiments were begun. Consequently diets that contained 0.75, 0.50, and 0.25 per cent UO_2F_2 were administered. The highest of these three levels (0.75 per cent) at first appeared to be tolerated; however, the rats failed to gain weight, and all died in the first 2 months of the experiment.

As this experiment went on, it became clear that both 0.50 and 0.25 per cent in the diet produced marked depression in growth. The rats receiving 0.50 per cent weighed 100 g less on the average than the controls by the end of the second month and maintained this differential thereafter for the remainder of the year. The rats fed 0.25 per cent averaged 30 to 40 g less than the controls after the second month for the balance of the year. It was apparent that 0.50 per cent represented something like the maximum tolerated chronic dosage. It was also apparent that 0.25 per cent was considerably above the minimum level to give detectable growth depression.

Experiments 2 and 3 were set up to find, if possible, a maximum amount that would give no change in growth rate, and the minimum amount that would consistently give evidence of poisoning shown by body weight. The intermediate levels of 0.01, 0.05, 0.1, and 0.15 per cent were studied in male rats; 0.01 and 0.05 per cent in female rats. There was no doubt that rats receiving 0.01 per cent in the diet had the same body weight as the control rats, male and female. Rats fed 0.05 per cent had either the same weight as the control rats (Experiment 2) or a greater weight (Experiment 3). Male rats fed 0.1 per cent UO_2F_2 consistently had lower average body weights than the control rats. These weights averaged less than 10 g lower through the first 6 months and 10 to 20 g lower during the balance of the year. There is good reason to believe that this represents the critical level, the minimum amount that gives a detectable growth depression on chronic feeding.

Confirmatory evidence is found in the body weights of the male rats fed 0.15 per cent. These rats averaged 10 to 20 g less than the controls during the first 6 months, and 30 to 40 g less during the remainder of the year. The response to 0.15 per cent is therefore interposed between 0.25 and 0.1 per cent, respectively.

Mortality. In male and female rats fed 0.75 per cent UO_2F_2, 100 per cent mortality was achieved by the end of the second month. In rats fed 0.50 per cent the mortality was 57 per cent in the males by the end of 12 months, and 33 per cent in the females at the same time. In the 16 other groups of rats no significant differences in mortalities were observed. The 155 control male rats exhibited a 12 per cent mortality by the end of a year; the 165 male rats fed amounts of UO_2F_2 up to and including 0.25 per cent also exhibited a 12 per cent mortality in the same period (see Table 7.8). The 140 female rats showed a

Table 7.8—Mortality of Rats Fed Various Uranium Compounds in the Diets for a Period of 2 Years

Compound	No. of rats	Mortality, %			No. of rats	Mortality, %		
		4 months	8 months	12 months		16 months	20 months	24 months
				Males				
Controls	155	1	8	12	105	27	46	64
Through 0.25% UO_2F_2	165	2	4	12	65	29	63	82
Through 2% $UO_2(NO_3)_2.6H_2O$	200	4	10	16	125	28	49	68
Through 20% UF_4	45	0	2	4	45	18	40	60
Through 20% UO_2	45	2	4	11	45	18	44	67
				Females				
Controls	140	2	3	9	90	23	52	61
Through 0.25% UO_2F_2	90	1	1	6	90	18	29	34
Through 2% $UO_2(NO_3)_2.6H_2O$	235	6	8	11	60	30	57	72
Through 20% UF_4	45	2	9	20	45	31	65	78
Through 20% UO_2	45	0	7	9	45	24	42	60

mortality of 9 per cent at the end of 12 months; the 90 female rats (0.25 per cent and less in the diet) showed a mortality of 6 per cent. It is interesting to observe that diets which produced unquestionable evidence of growth depression had no perceptible effect on mortality during the year period.

4.2 Uranyl Nitrate, Growth Effects. In the 30-day feeding experiments, extensive mortalities had been observed in groups of rats fed, respectively, 20, 10, 5, and 3 per cent uranyl nitrate hexahydrate. Some mortality (7 to 16 per cent) had been found in rats fed 2 per cent uranyl nitrate hexahydrate. The latter fatalities occurred during the second week of feeding at the time when the typical uranium-poisoning deaths occurred following intraperitoneal administration of uranium compounds. The surviving rats at the end of 30 days were gaining weight and appeared to be in good shape. Consequently 2 per cent was assumed to represent a maximum level at which groups of rats might be fed uranyl nitrate hexahydrate chronically.

Three experiments were set up to study the effects of chronic feeding of uranyl nitrate hexahydrate in rats (Tables 7.9 and 7.10). The first of these comprised four groups of 15 male rats and 20 female rats each fed, respectively, 0, 0.01, 0.05, and 0.1 per cent uranyl nitrate hexahydrate in the diets. The second experiment comprised five groups of rats, 20 male and 25 female per group, fed 0, 0.1, 0.5, 1.0, and 2.0 per cent uranyl nitrate hexahydrate in the diet. In the third experiment, there were four groups of rats, 25 male and 25 female each, fed 0, 0.1, 0.5, and 2.0 per cent.

Since uranyl nitrate has been so widely studied, certain growth curves have been selected that illustrate the major aspects of the growth observed during the first year of the 2-year studies of each level of the compound. These are shown in Figs. 7.12 and 7.13. The average body weights for the control rats and those receiving 0.1, 0.5, and 2.0 per cent in their diets (both male and female rats) have been plotted. The outstanding fact shown is the characteristic growth curves of each group. These curves are not perfectly smooth; there are frequent minor fluctuations and occasional major fluctuations. The sources of these variations, in general, are unknown. Some of the possible factors might be:

1. A sudden change in the weather, an extraordinarily high or low room temperature during a few hours or a day or so.
2. A mild or a more severe epidemic of "sniffles" or diarrhea—never serious in our colony but a plague of all chronic experiments.
3. A single case of labyrinthitis or an isolated spontaneous tumor, which provided opportunity for abnormal weight changes with abrupt differences in average weights when such an animal succumbed or was sacrificed.

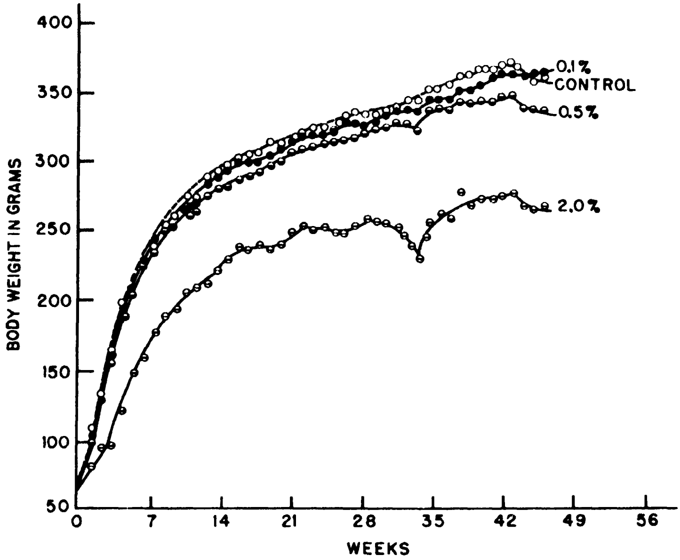

Fig. 7.12—Growth curves for groups of male rats fed various dietary levels of uranyl nitrate hexahydrate for about 1 year.

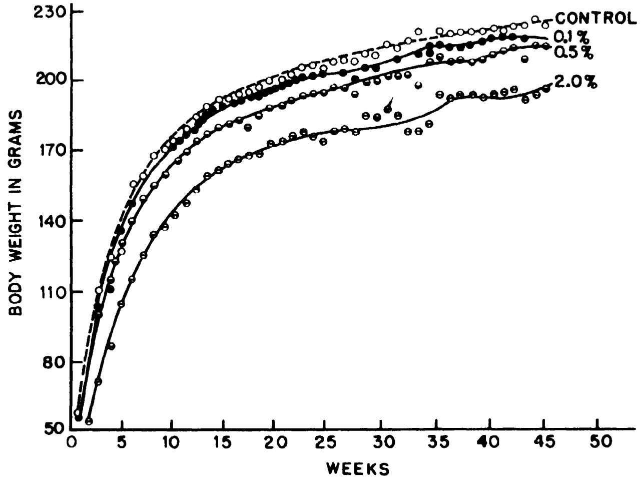

Fig. 7.13—Growth curves for female rats fed various levels of uranyl nitrate hexahydrate in the diet for 1 year.

Table 7.9 — Average Difference in Body Weight of Groups of Male Rats Fed Uranyl Nitrate Hexahydrate Compared with the Average Weight of the Control Groups

Exptl. group*	Amount in diet, %	No. of rats per expt.	0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo	8 mo.	12 mo.	16 mo.	20 mo.	24 mo.	Comments on growth
			Body wt., g												
1	None	15	61	168	254	304	327	353	366	360	385	390	400	377	Normal
2	None	20	48	157	239	277	313	329	347	375	396	411	411	395	Normal
3	None	25	70	195	253	290	302	320	328	347					Normal
			Weight difference from controls, g												
1	0.01	15	+1	−7	−15	−16	−12	−16	−16	−18	−26	−25	−36	+96	Consistently grew less than controls
1	0.05	15	+2	−1	−5	−4	+5	+1	0	+17	+6	+41	+52	+62	Consistently outgrew controls
1	0.1	15	+1	−10	−19	−21	−39	−35	−41	−21	−36	−23	+39	+2	Consistently grew less than controls
2	0.1	20	+1	−12	−23	−21	−17	−8	−5	−13	−32	−12	−2	−6	Consistently grew less than controls
3	0.1	25	+5	+3	+11	+3	+5	+5	+9	+12					Same as controls
2	0.5	20	0	−8	−17	−26	−18	−9	−11	−14	−37	−11	+1	+57	Consistently grew less than controls
3	0.5	25	0	−4	+1	−7	−8	−8	−10	−7					Consistently grew less than controls
2	1.0	20	−1	−20	−45	−41	−36	−31	−35	−38	−42	−33	−29	−27	Marked depression of growth
2	2.0	20	0	−38	−66	−72	−73	−64	−60	−71	−74	−80	−54	−26	Marked depression of growth
3	2.0	25	0	−72	−57	−67	−62	−70	−71	−86					

*To identify the individual experiments the control and experimental groups are given the same integer.

Table 7.10—Average Difference in Body Weight of Groups of Female Rats Fed Uranyl Nitrate Hexahydrate Compared with the Average Weight of the Control Groups

Exptl. group*	Amount in diet, %	No. of rats per expt.	0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	8 mo.	12 mo.	16 mo.	20 mo.	24 mo.	Comments on growth
							Body wt., g								
1	None	20	51	126	176	193	204	208	215	220	229	245	259	232	Normal
2	None	25	91	151	181	195	199	207	215	226	237	260			Normal
3	None	25	59	156	175	191	200	207	208	221					
							Weight difference from controls, g								
1	0.01	20	−1	−2	−1	−1	+1	+3	+3	+6	+8	+8	+18	+44	Within normal limits
1	0.05	20	−2	−1	−3	−3	0	−3	−1	0	+2	−16	−21	+32	Within normal limits
1	0.1	20	0	+3	+1	+2	+3	+6	+5	+5	+18	+15	+13	+36	Within normal limits
2	0.1	25	−1	−3	−2	−4	−6	−2	−4	−3	+6	−6			Same as controls
3	0.1	25	−3	−9	−2	−2	−4	−5	−7	−7					Same as controls
2	0.5	25	0	−5	−11	−9	−10	−10	−9	−16	−15	−26			Consistently grew less than controls
3	0.5	25	−1	−17	−13	−11	−12	−13	−14	−12					Consistently grew less than controls
2	1.0	25	−1	−10	−14	−19	−18	−19	−21	−26	−20	−38			Consistently grew less than controls
2	2.0	25	0	−14	−19	−33	−26	−29	−27	−34	−37	−55			Marked depression of growth
3	2.0	25	−2	−41	−33	−30	−27	−31	−30	−28					Marked depression of growth

*To identify the individual experiments, the control and experimental groups are given the same integer.

The dietary percentages given in the figures show a progressive effect in growth depression, i.e., the addition of 2 per cent uranyl nitrate hexahydrate to the diet markedly reduces the growth curve, 0.5 per cent is clearly effective in maintaining growth curves below those of the controls, and 0.1 per cent in the diet influences the growth curves so little that there may be some doubt as to whether this is a real change. Similar selections of curves could be made for UO_2F_2; however, not at the same dietary percentages. The final point that should be made in connection with these curves is the close paralleling of the various curves, particularly in the second half year of the feeding period. This phenomenon was observed in most of the experiments undertaken.

When male and female rats were fed diets containing 2 per cent uranyl nitrate hexahydrate, a marked depression in growth rate was observed. For the male rats the growth depression amounted to 60 to 70 g by the second or third month. This reduced weight was maintained during the remainder of the year. For the female rats receiving 2 per cent uranyl nitrate hexahydrate, the body weights were about 30 g less than the average of the control rats from the third to the twelfth months.

One per cent uranyl nitrate hexahydrate also produced a marked growth depression amounting to 30 or 40 g on the average in the male rats and around 20 g for the females.

Male and female rats fed 0.5 per cent uranyl nitrate hexahydrate consistently grew less than the litter-mate controls. In the male rats this depression amounted to 10 to 25 g, in the female rats 10 to 17 g. This level apparently is the lowest amount that regularly gives a measurable loss in growth.

Male and female rats fed 0.1 per cent uranyl nitrate hexahydrate sometimes weighed more and sometimes weighed less, on the average, than the corresponding control rats. In most of the groups these differences were not of very large order (± 5 g). In one group of males the growth was depressed 20 to 40 g consistently. However, another group of males with equal consistency outweighed the controls by 5 to 12 g. Two groups of female rats consistently weighed a little less than the litter-mate controls, but a third group weighed a little more than the controls. During the time these experiments were going on, these contradictory trends introduced a perpetual uncertainty as to whether 0.1 per cent uranyl nitrate hexahydrate did or did not influence the growth processes of rats. In the monthly reports this uncertainty was manifested by constantly varying opinions. It is reasonable now to take 0.1 per cent as the highest level in the diet that can be fed with no injurious effect on growth. The region between 0.1 and 0.5 per cent constitutes a minus-plus zone of injury.

Male and female rats were fed diets containing 0.05 and 0.01 per cent uranyl nitrate hexahydrate. In each case there is no reason to doubt the absence of a physiologically detectable effect. The female rats on both diets weighed almost exactly the same as their respective control groups during the entire 1-year period. The male rats fed 0.01 per cent weighed less than the control group consistently and markedly (10 to 20 g); however, the male litter mates in the same experiment, which received 0.05 per cent during the last 8 months, outgrew the control groups. This clearly points to the random nature of the growth effect of feeding the lesser quantities.

Mortality. Twenty-six groups of rats, 15 to 25 rats per group, were fed various amounts of uranyl nitrate over a 2-year period. During the first year of the experiment the mortalities in the various groups ranged from 0 as the lowest figure to 35 per cent as the highest figure. There was no demonstrable dependence of mortality on dosage in the diet levels studied. During the first 2 weeks on the 2 per cent diet, a possible exception to this statement was noted. There were always a few deaths among the young rats, perhaps as great as 4 to 6 per cent of the group. This mortality apparently was of the weaker rats, which would not have survived the first year of the study. See Table 7.8, where all the results have been grouped. Among the 200 male rats fed uranyl nitrate hexahydrate at levels up to and including 2 per cent, 16 per cent had died at the end of the year. This compares favorably with the 12 per cent mortality among 155 control rats. Two hundred thirty-five female rats on the same dietary levels exhibited a mortality of 11 per cent at the end of 12 months that is nearly identical with the 9 per cent mortality exhibited by the 140 control rats. There is evidence that inclusion of up to 2 per cent uranyl nitrate hexahydrate in the diet was without effect on the mortality of rats over a period of 12 months, even though at the higher levels a marked reduction in growth was observed.

Pathology. Kidney injury was found in the rats at 0.5 and 2 per cent dietary levels (see Chap. 4 for details). No renal injury occurred at the 0.1 per cent dietary level.

4.3 Uranium Tetrafluoride, Growth Effects. A 30-day feeding experiment in which UF_4 was added to the diets of rats at levels of 20, 2, and 0.5 per cent gave evidence of the low degree of toxicity of this compound. No rats died and no rats exhibited a loss of body weight when fed diets containing 0.5 and 2 per cent UF_4, respectively. When 20 per cent UF_4 was fed, the average body weights of the rats were considerably less than those of the controls; this reduction amounted to 15 to 50 g in the male rats and 5 to 20 g in the female rats. Of 38 male rats studied, none died, and, of 37 female rats, one died.

Table 7.11 — Average Difference in Body Weight of Groups of Male Rats Fed Uranium Tetrafluoride Compared with the Average Weight of the Control Groups

Amount in diet, %	No. of rats per expt.	0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	8 mo.	12 mo.	16 mo.	20 mo.	24 mo.	Comments on growth
		Body wt., g												
None	15	50	146	237	277	301	325	334	339	362	385	388	373	Normal
		Weight difference from controls, g												
0.5	15	−2	0	+3	+9	+10	+9	+11	+14	+15	+1	−15	−4	Consistently outweighed controls
2.0	15	−2	−1	+3	+7	+10	+10	+6	+10	+2	−19	+15	+42	Consistently outweighed controls
20.0	15	+2	−13	−10	−2	−6	−8	−18	−29	−35	−34	−24	−24	Marked depression of growth

Table 7.12 — Average Difference in Body Weight of Groups of Female Rats Fed Uranium Tetrafluoride Compared with the Average Weight of the Control Groups

Amount in diet, %	No. of rats per expt.	0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	8 mo.	12 mo.	16 mo.	20 mo.	24 mo.	Comments on growth
		Body wt., g												
None	15	55	116	153	174	187	198	205	216	232	243	227		Normal
		Weight difference from controls, g												
0.5	15	−2	+2	+4	+5	+5	+10	+8	+15	+14	−5	+117	All dead	Consistently outweighed controls
2.0	15	−1	+3	+6	+6	+4	+5	+4	+6	−6	−22	−19		Same as controls
20.0	15	−1	−13	−13	−8	−5	−3	−5	−7	−16	−33	+19		Consistent depression of growth

There seemed to be every prospect for maintaining rats for a period of 1 year on the same widely spaced levels used in the 30-day experiments. Selected data on the body weights of rats given such diets for 24 months are shown in Tables 7.11 and 7.12. Groups of 15 male and 15 female rats each were placed on these diets when their initial body weights were 50 to 55 g.

Twenty per cent UF_4 in the diets of both male and female rats consistently produced a clearly indicated depression of growth. During the latter half of the first year period, growth depression amounted to 20 to 35 g in the case of the male rats, and 5 to 15 g in the case of the female rats. As we have seen in the studies of UO_2F_2 and uranyl nitrate, such depressions of growth weight did not represent a severe degree of poisoning. It is difficult to estimate at what percentage in the diet UF_4 would fail to show any effect. Certainly 20 per cent cannot be taken as the minimum that can be given with detectable injury. Perhaps 10 per cent would be a better approximation.

Male and female rats fed 2 per cent UF_4 showed no signs of injury in the growth pattern; the male rats consistently outweighed the controls by 5 or 10 g. The female rats weighed essentially the same as the control group.

Male and female rats fed 0.5 per cent UF_4 gave no evidence of toxic effect; on the contrary, both male and female groups consistently outweighed the control groups by 10 or 15 g. It is difficult to say from these data where the plus or minus zone of toxicological effect lies. It has been demonstrated that 2 per cent UF_4 is without effect and that 20 per cent has a clear-cut effect. This is a broad zone in which the plus and minus area lies.

Mortality. Eight groups of rats, male and female combined, suffered mortalities of 0 to 22 per cent during the first year of the experimental period. For some unknown reason, the 45 males had very low mortalities, amounting to only 4 per cent at the end of 12 months as compared to 12 per cent in the control rats (see Table 7.8). On the other hand, the female rats showed a surprisingly high mortality. Twenty per cent of the 45 rats died as compared with 9 per cent of the control rats.

4.4 Uranium Dioxide, Growth Effects. One-month feeding experiments were carried out in which rats received diets containing 20, 2, and 0.5 per cent UO_2. No rats died in any of the groups, and there was no single group of the 12 observed that gave evidence of a loss of growth. In every case, except one, the average weights were nearly identical with those of the control groups. The exception was one group of male rats fed 20 per cent, in which the average weight was 27 g greater than that of the control group.

Table 7.13—Average Difference in Body Weight of Groups of Male Rats Fed Uranium Dioxide Compared with the Average Weight of the Control Groups

Amount in diet, %	No. of rats per expt.	0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	8 mo.	12 mo.	16 mo.	20 mo.	24 mo.	Comments on growth
		Body wt., g												
None	15	49	179	284	320	338	340	364	376	411	401	440	254	Normal
		Weight difference from controls, g												
0.5	15	+2	+10	+15	+16	+15	+35	+20	+18	+13	+25	−7	+165	Consistently outgrew controls
2.0	15	+4	+13	+12	+13	+2	+16	+9	+10	−2	+2	−50	+146	Same as controls
20.0	15	+1	−2	−1	−2	−1	+1	−7	−3	−9	−1	−57	+131	Same as controls

Table 7.14—Average Difference in Body Weight of Groups of Female Rats Fed Uranium Dioxide Compared with the Average Weight of the Control Groups

Amount in diet, %	No. of rats per expt.	0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	8 mo.	12 mo.	16 mo.	20 mo.	24 mo.	Comments on growth
		Body wt., g												
None	15	52	131	180	198	209	214	220	226	242	254	256	262	Normal
		Weight difference from controls, g												
0.5	15	−1	+2	+4	+2	+2	0	−2	0	+2	−1	−18	+3	Same as controls
2.0	15	0	−2	+2	+1	+1	0	+2	+1	0	+8	+16	+26	Same as controls
20.0	15	+1	+3	+5	+7	+7	+9	+6	+15	+28	+32	+58	+60	Consistently outgrew controls

Using the information obtained in the 30-day tests, it seemed that there was no reason to suspect that rats fed 20 per cent UO_2 should not survive for a year. Groups were thereupon given 0.5, 2, and 20 per cent UO_2. Fifteen male and fifteen female rats were fed at each level; the initial body weights varied from 49 to 53 g. As is shown in Tables 7.13 and 7.14, the growth curves are normal in every case.

Male and female rats fed 20 per cent UO_2 weighed the same as or more than the respective control rats. The male rats frequently weighed a few grams less than the controls, whereas the female rats consistently outweighed the controls by 7 to 28 g. These differences are regarded as occurring by chance.

Male and female rats fed 2 per cent UO_2 weighed slightly more than the controls in the case of the males and almost precisely the same as the control rats in the case of the females.

Male and female rats fed 0.5 per cent UO_2 grew well. The males outgrew the controls by 10 to 30 g, whereas the females had average body weights almost identical with those of the control rats. These data did not establish the largest amount that can be fed without detectable injury. It should be noted that there are few substances sufficiently inert to show no effect on growth weights following the inclusion of 20 per cent in the diet. All that has been shown is that 20 per cent UO_2 in the diet does not reduce the growth weight.

Mortality. In the eight groups of rats fed UO_2, mortalities were observed varying from 0 to 15 per cent. The over-all mortality of the 45 male rats was 11 per cent, which is almost identical with the 12 per cent mortality of the control rats (see Table 7.8). The 9 per cent mortality in the 45 female rats was exactly the same as the values found in the control rats.

4.5 Discussion. Despite the minor fluctuations that were observed in random occurrence in all the growth curves, there was a general consistent pattern of increase in which the most rapid increment of growth occurred in the first 3 months. A slower growth rate was then established, which continued for the remainder of the year. The tendencies to growth depressions in general were established early, i.e., in the first 2 or 3 months, and it is interesting to note the regularity with which the differences, once established, were maintained during the second half year. Average weight-depression figures at the sixth month and the ranges of weight depressions in the various months from 6 to 12 are given for both male and female rats in Table 7.15. The consistency of differences for the groups may be seen individually by referring to Tables 7.6, 7.7, and 7.9 to 7.14.

In Table 7.15 it is observed that with increase in dietary percentage the consistent weight depression increased. It is interesting to compare the magnitude of this depression for the three compounds that produced a depression. This comparison has been carried out visually in Fig. 7.14. In this figure the average depression of weight is indicated as the ordinate, and the logarithm of the percentage of the various compounds is indicated along the abscissa. The heavy-lined blocks indicate the data for male rats, the light-lined blocks the data for female rats. These values have been selected because the weight depressions are closely comparable numerically and because they emphasize the marked difference in dietary percentages that produced

Table 7.15—Data on Average Body Weights in Second 6-month Period

Compound	% in diet	Weight depression			
		Males		Females	
		6-month average	12-month range	6-month average	12-month range
UO_2F_2	0.10	15	8-23		
	0.15	35	16-45		
	0.25	40	32-47	13	9-15
	0.25	30	20-43	18	14-21
	0.50	110	101-118	42	35-50
$UO_2(NO_3)_2.6H_2O$	0.5	23	11-37	13	9-16
	0.5	15	7-27	11	9-14
	1.0	40	35-43	24	20-27
	2.0	68	60-76	32	27-37
	2.0	87	71-96	28	27-30
UF_4	20.0	26	18-35	9	3-16

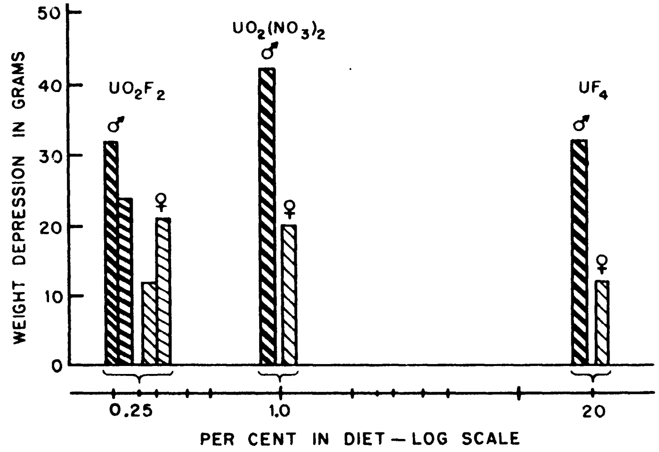

Fig. 7.14—Weight depression in rats fed various uranium compounds.

them. At the left of the figure, weight reductions of 24 to 32 g in male rats and 12 to 21 g in female rats are shown above the value of 0.25 per cent UO_2F_2 in the diets. In the center of the figure, weight depressions of 42 g for male rats and 20 g for female rats are associated with 1 per cent uranyl nitrate hexahydrate in the diets. At the right of the figure, weight losses of 32 g in the male rats and 12 g in the female rats are placed above the value of 20 per cent UF_4 in the diets. This gives a striking graphic comparison of the marked differences

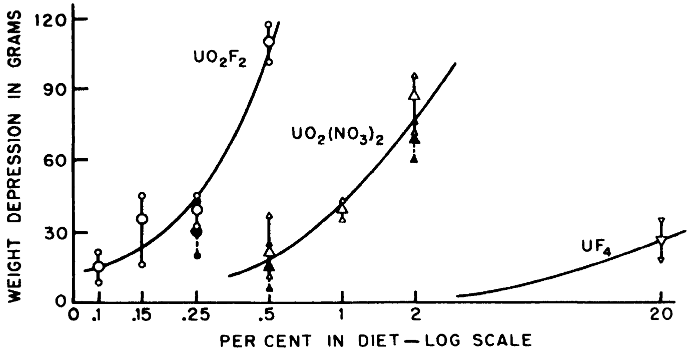

Fig. 7.15—Average weight depression, sixth to twelfth months, in groups of male rats fed various uranium compounds. Range of weight depression within each group is indicated. Large symbols denote average values. Solid symbols represent results from repeated experiments.

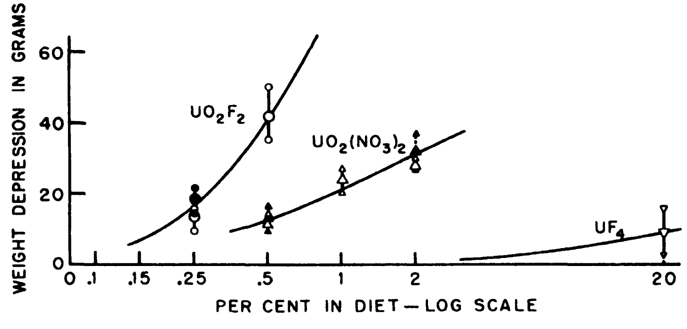

Fig. 7.16—Average weight depression, sixth to twelfth months, in groups of female rats fed various uranium compounds. Range of weight depression within each group is indicated. Large symbols denote average values. Solid symbols represent results from repeated experiments.

in the amounts of these three compounds required to produce comparable depression in growth at the end of the 12-month period.

The consistency of the response has been gratifying. This consistency may be shown even in groups of animals set up in experiments begun months apart and fed widely varying amounts of the various compounds. This consistency is shown in Figs. 7.15 and 7.16, where the average weight depressions for the sixth to twelfth months are plotted as ordinates against the logarithm of the percentages in the diets. The ranges of the average weight depressions during these 6-month periods are indicated by the length of the line above and below the respective averages. The values for male and female rats are plotted separately. In Fig. 7.15 at the left are shown grouped about a curved line the average weight depressions of rats fed various diets containing from 0.1 to 0.5 per cent UO_2F_2. When it is considered that these include all the observations on all the groups, the indicated consistency is striking. The similar data for female rats are given in Fig. 7.16. Somewhat more variation was observed in the male rats receiving 0.5 per cent UO_2F_2 than is the case with the female rats. In the center of Figs. 7.15 and 7.16 the data are given for rats fed 0.5 to 2 per cent uranyl nitrate hexahydrate. Again, a curvilinear tendency is shown for both male and female rats. The data for UF_4 are given at the right-hand side of Figs. 7.15 and 7.16 and indicate only the lesser response for this less toxic compound. It is interesting to note the evidence of reproducibility that these figures represent when applied to the groups of experiments taken as a whole. Evidence here may be seen for reproducibility of results, for regularity of response as measured by the growth depression resulting from logarithmic increments in the amounts of toxic agents, and, finally, for a direct visual comparison of the relative toxicities of the three compounds. The curves for UO_2F_2, the most toxic, are at the left of the figures in the region of low doses; the curves for uranyl nitrate, of intermediate toxicity, are in the center of the figures at medium doses; and the curves for UF_4, the least toxic, are at the right of the figures in the region of high doses.

4.6 Breeding Test. There had been several findings that raised the question of an effect on ovaries or testes of "chronic" uranium administration. A simple experiment was set up in which two groups of animals were observed: (1) pairs of control rats fed the stock diet and (2) pairs of experimental rats fed a diet containing 2 per cent uranyl nitrate hexahydrate. Fifty males and 50 females were used in each of the two groups. The growth curves are shown in Fig. 7.17. It is plain that the growth rate was noticeably depressed for both male and female rats fed 2 per cent uranyl nitrate hexahydrate as compared to their respective controls. The rats were allowed to mate; the females were separated from the males late in pregnancy. The litters were removed on the day of birth, and the females were returned to the males at that time. The number of litters and the number of young per litter were recorded. The young were analyzed as a group for total uranium content by the fluorophotometric method.

The outstanding result of the experiment was a marked decrease in total number of litters borne by the experimental rats as compared to the control rats. During the 195 days that the dietary regimen was continued, the 50 pairs of control rats had borne 249 litters, whereas the 50 pairs of rats fed 2 per cent uranyl nitrate hexahydrate had borne 135 litters, a ratio of nearly 2 : 1 in favor of the control rats.

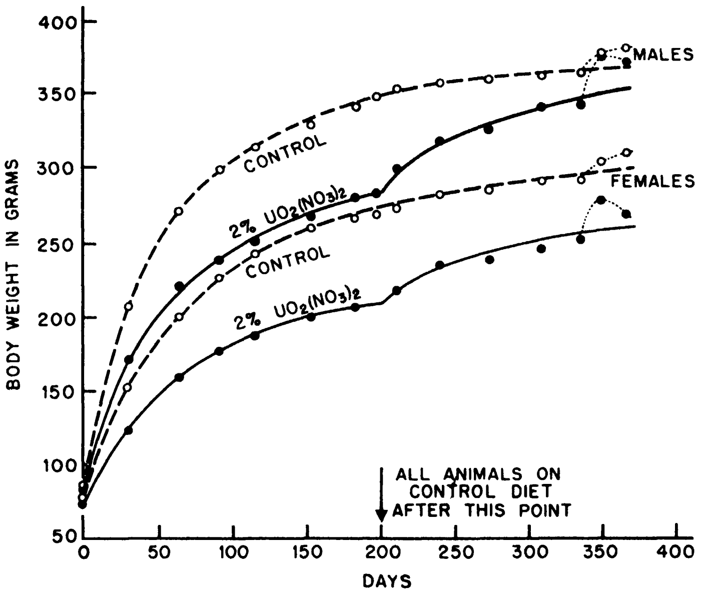

Fig. 7.17—Growth curves for rats fed 2 per cent uranyl nitrate hexahydrate for 7 months. Control diet for 4 months.

This difference in the number of litters was established from the first month of the experiment and depended entirely on the fact that more females bore litters on the control diet. The females that had litters on both diets had them at approximately the same rate, and had about as many young per litter on the average.

After 195 days on diet the numbers of litters were as given in the table below.

Group	Number of litters 1st	 2d	 7th
Control	43	39	19
Uranyl nitrate hexahydrate, 2%	30	21	3

These figures indicate a slight retardation in the frequency of litters of experimental rats.

The average number of young per litter is given in the table below.

Group	Average number of young per litter 1st	 2nd	 3rd	 4th	 5th
Control	7.9	9.9	9.7	9.6	8.5
Uranyl nitrate hexahydrate, 2%	7.8	7.8	7.5	8.5	8.2

The reduction in the average number of young per litter may be some evidence of toxic effect of the uranyl nitrate.

The decrease in the number of litters described above may be attributed in some measure to the same process that produced the depression in growth seen in Fig. 7.17. It has been established by careful physiological studies that the growth rate is an accurate reflection of the food intake. Consequently it is possible to interpret the depression of growth of the animals fed 2 per cent uranyl nitrate hexahydrate as a partial inanition. It is also well known that inanition produces a serious interference with reproductive activity in many animals. Consequently the decrease in the number of litters may be interpreted as a secondary effect of the quasi-inanition induced by the uranium poisoning.

After 195 days on the diet the experimental rats were shifted to the stock fox-chow-meal diet. It was intended to find out by this change whether the growth of the experimental rats had been permanently retarded and whether any improvement in the number of litters born might thereafter occur. The rats were maintained on this regimen for the remainder of a year. As may be seen from Fig. 7.17, there was a prompt change in the direction of the growth curves for both male and female rats. An upswing in growth began and led, in the case of the male rats, to body weights still somewhat below those of the control rats at the end of 12 months and to average weights that differed by 25 to 40 g in the case of the female rats. These figures should be contrasted with the difference of 65 g in the average male body weights and of 55 g in the average female body weights immediately before the change in diets. It is therefore shown that the growth impetus was only retarded and not completely destroyed by the continued ingestion of the diet containing 2 per cent uranyl nitrate hexahydrate. The rats still possessed the ability to regain approximately their normal body weight after a span of some 5 months.

It is also interesting to compare what happened to the number of litters born. At the end of the year the 50 pairs of controls had borne 367 litters. The 50 pairs of rats receiving 2 per cent uranyl nitrate hexahydrate in the diet had borne 217 litters. The over-all productivity had not changed much as compared with the rate up to 195 days. The control rats had borne 1.7 times as many litters at the end of the year as compared to 1.85 at the end of the 7-month experimental feeding period. In the 5-month period between the change to the stock diet and the termination of the experiment, the controls had 104 litters; the experimentals had 72 litters. Thus the control rats had 1.44 times as many litters as the experimental rats. This may indicate a slightly increased birth rate in experimental litters. The female rats from the experimental group that had failed to have litters in the early part of the experiment continued not to have litters. For example, at the end of the year the numbers of litters were as follows:

	Numbers of litters		
Group	7th	10th	12th
Control	34	16	2
Uranyl nitrate hexahydrate, 2%	18	5	0

In the 5 months in which both groups received the stock diet, the control males increased 17 g in body weight on the average, whereas the experimental males increased 60 g. The control females increased 22 g in body weight in the same period, whereas the experimental rats increased 43 g. These figures show clearly the spurt in body weight that followed the removal of the rats from the toxic diet.

The breeding test has shown that the animals fed at a level that produced a marked depression of growth rate also produced a clear-cut depression in rate of reproduction as measured by the number of litters and, to some extent, by the number of young per litter. This difference in number of litters arose from the larger number of females on the experimental diet that did not bear litters at all. After

7 months on the 2 per cent uranyl nitrate hexahydrate diet, when the animals were placed on the stock diet, there was prompt resumption of growth and some indication of an increased birth rate.

At the beginning of the fourth month of the experiment a check was begun on the regularity of the estrous cycles of the control females, and also of the females fed 2 per cent uranyl nitrate hexahydrate, in relation to their regularity of mating. Such determinations were made by Randall for a period of 3 months by daily microscopic studies of vaginal smears, with the following results:

Matings	No. of matings		Estrous cycle			
			Regular		Irregular	
	Control	$UO_2(NO_3)_2$ $.6H_2O$, 2%	Control	$UO_2(NO_3)_2$ $.6H_2O$, 2%	Control	$UO_2(NO_3)_2$ $.6H_2O$, 2%
Regular	41	19	41	19		
Irregular	0	8	0	7	0	1
None	4	17	2	4	2	13
Totals	45	44	43	30	2	14

It may be seen that of the four control females that had no matings during the 3-month period, two had irregular estrous cycles. Of the 17 females on a diet of 2 per cent uranyl nitrate hexahydrate that had no matings during the same period, 13 had irregular estrous cycles. It may be that such irregularity in the estrous cycles of the females fed 2 per cent uranyl nitrate hexahydrate in this experiment was due to a reduction in the food intake (a partial inanition) rather than to a direct toxic effect of the uranium.

4.7 Summary. 1. Groups of male and female albino rats were fed diets containing various levels of UO_2F_2, uranyl nitrate, UF_4, and UO_2 for a period of 1 year.

2. The minimum dietary percentages of the four compounds required to produce unquestionable evidence of growth depression are as follows:

Compound	Minimum giving growth depression, %	Minimum giving increase in mortality during 1 year, %
UO_2F_2	0.1	0.5
$UO_2(NO_3)_2$ $.6H_2O$	0.5	3.0
UF_4	<20	?
UO_2	>20	?

3. Most of the diets failed to produce an increase in mortality over that observed in 295 control rats. The minimum percentages giving increases in mortality during the year are shown in the above table.

4. For three of the compounds, namely, UO_2F_2, uranyl nitrate, and UF_4, increasing dietary percentages gave evidence of toxic effects by depressed growth curves. The curves, once established, remained parallel in the second 6-month feeding period.

5. Some concept of the relative toxicities of the compounds may be obtained by considering the dietary percentages that produced equal body-weight depressions (12 to 30 g average less than the control body weights). The compounds and percentages in the diets that produced this effect are as follows: UO_2F_2, 0.25 per cent; uranyl nitrate hexahydrate, 1.0 per cent; UF_4, 20 per cent. Twenty per cent UO_2 in the diets failed to influence the growth.

6. In a breeding experiment, 50 pairs of control rats had 249 litters, while 50 pairs of rats fed 2 per cent uranyl nitrate hexahydrate had 135 litters during a 7-month period. The difference is largely attributable to the fact that fewer females had litters in the experimental group.

7. When the rats in the breeding experiment had been 7 months on the experimental regimen, those fed a diet containing 2 per cent uranyl nitrate hexahydrate were placed for 5 months on the stock ration. The prompt resumption of growth enabled the experimental groups almost to equal the control groups in body weight at the end of the experiment. Thus the growth impetus was only retarded by ingestion for 7 months of a diet containing 2 per cent uranyl nitrate hexahydrate.

8. Two of four control female rats that had no matings from the 4th to 6th month, inclusive, had irregular estrous cycles; 13 of 17 female rats on a diet of 2 per cent uranyl nitrate hexahydrate that had no matings during the same period also had irregular estrous cycles.

5. TWO-YEAR FEEDING EXPERIMENTS, RATS

The ingestion experiments were reviewed at the end of the first year, and the decision was made to continue the chronic feeding experiments through the second year, provided the rats survived. As has been indicated, one or more groups had shown growth and other characteristics indistinguishable from the control groups during the first year. In other groups of rats sufficient toxic material had been fed to establish a body weight certainly less than the average for the control rats.

During the second year (Tables 7.6, 7.7, and 7.9 to 7.14) the tendencies established in the second half of the first year were maintained with extraordinary consistency. This consistency is illustrated in the accompanying graph (Fig. 7.18), in which the growth curves for the first year are condensed at the left-hand side of the figure. Only the values for the control male and female rats and those fed 2 per cent

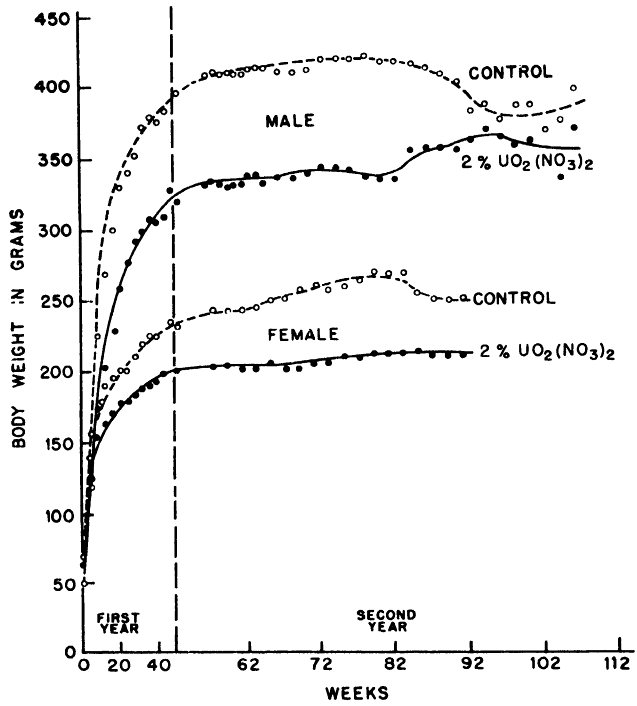

Fig. 7.18 — Growth curves for male and female rats fed 2 per cent uranyl nitrate hexahydrate in the diet for 2 years.

uranyl nitrate hexahydrate are included. The essential findings from the second-year study may be gained from considering this figure. The average body weights fluctuated somewhat but maintained average weight curves parallel to the abscissa in each case. For both males and females, the rats fed 2 per cent uranyl nitrate hexahydrate have weight curves set somewhat below those of the control rats, but maintained with a constant difference throughout the second year.

A close inspection of the Tables 7.6, 7.7, and 7.9 to 7.14 will show certain aberrations that are beyond the chance variations in average body weights. For example, in a group of male rats fed 0.01 per cent uranyl nitrate hexahydrate, the average body weight at the twentieth month was 36 g less than that of the corresponding controls. At the twenty-fourth month this had changed radically so that the average weight of the experimental rats was 96 g greater than the average for the control group. This exceptional example of variability was mirrored in lesser degree in numerous groups. The basis for such wide variations arises from the manner in which the averages were made. The body weights of each group were averaged, and the averages plotted at periodic intervals of 2 weeks. The survivors at the end of the 2-year period were sometimes a rather heterogeneous group, and consequently the averages became misleading. If a group contained one or two old fat males weighing 500 to 600 g and a few thin sickly males weighing 300 to 350 g, the death of one or two such animals might provide a change in the group average of 100 g or more. This became particularly noticeable in groups with a high mortality during the second year, in which only two or three rats survived. Consequently the wide changes in average body weight in the terminal months of the 2-year study are not alarming.

In order to obtain an orientation in the matter of growth curves, the various groups of control rats, male and female separately, were plotted on single graphs of body weight vs. age. These gave evidence of the remarkable concurrence of average body weight of the control groups of rats (Figs. 7.19 and 7.20).

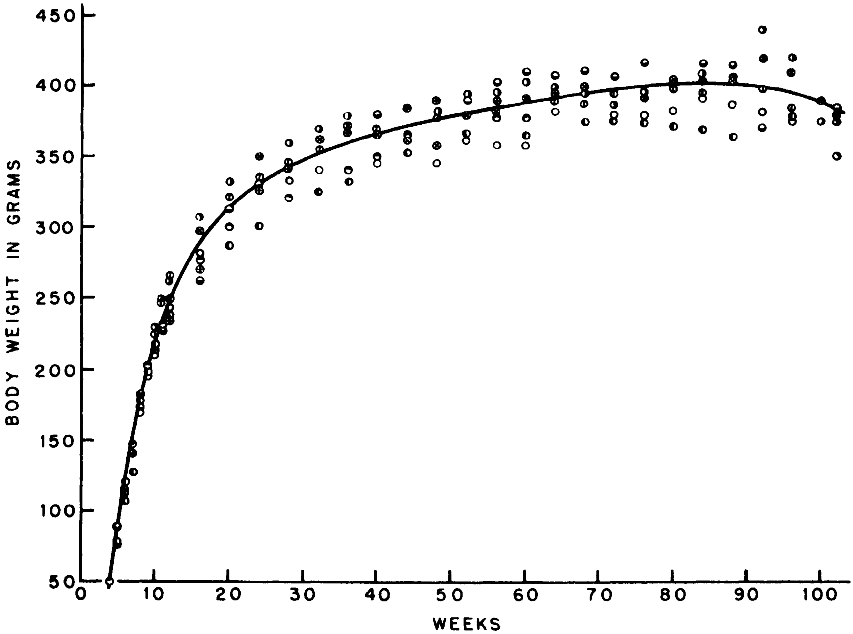

Fig. 7.19—Growth curves for groups of control male rats. Each symbol represents a different group.

During the early part of the first year, in the period of most rapid growth, perhaps up to 26 weeks, only small variations in body weight were found. These amounted to about 50 g for the various groups of male rats and about 20 g for the various groups of females. As may be seen from the growth curves these are the outside limits of differences between groups. A marked consistency was observed for the average of a given group; certain groups were among the lower average weights during most of the period, other groups were consistently among the heaviest. The variation increased somewhat in the second year but was marked only in the last 10 to 16 weeks. Averages were taken, and the control growth curves thus obtained were used in Figs. 7.21 and 7.22 to compare with the effects of various levels of UF_4, uranyl nitrate, and UO_2F_2, which were thus shown to have produced retardations in growth. For the male rats a marked reduction is given by 0.5 per cent UO_2F_2. This reduction is constant and noticeable during the entire 2-year period. Much less reduction is obtained by 2 per cent uranyl nitrate hexahydrate, although the wide differential is maintained consistently during the entire feeding period. It is interesting to see that a somewhat similar loss of weight is caused by

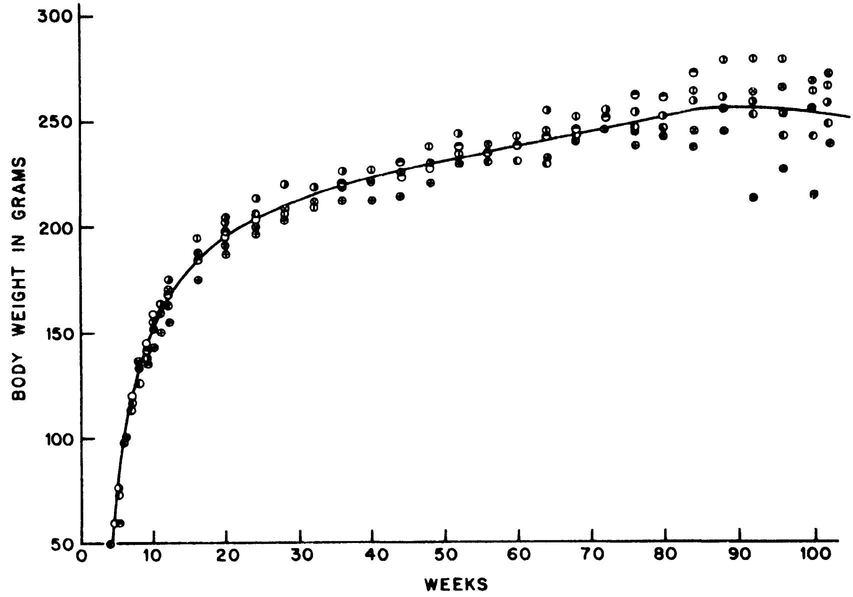

Fig. 7.20 —Growth curves for control female rats. Each symbol represents a different group.

feeding 20 per cent UF_4. This weight depression is somewhat less than is given by 2 per cent uranyl nitrate hexahydrate.

For the female rats the compounds just cited produced somewhat less effect, level for level, than was observed in the male rats. The 0.5 per cent UO_2F_2 consistently gave a reduction in growth that was

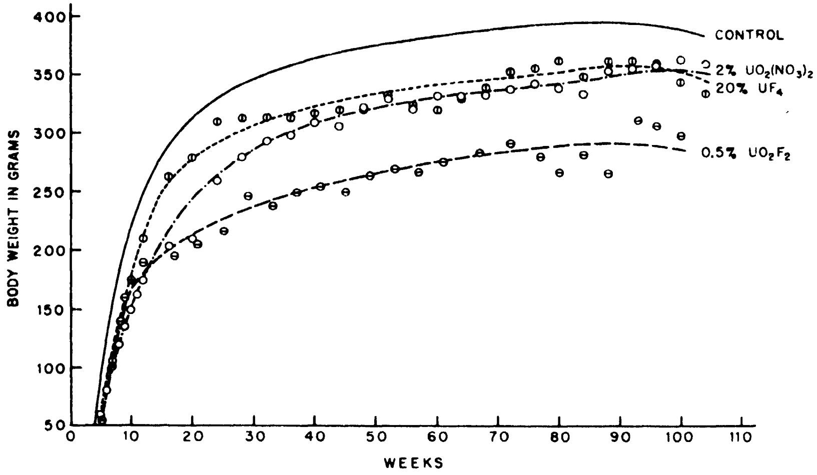

Fig. 7.21 —Growth curves for male rats fed certain uranium compounds.

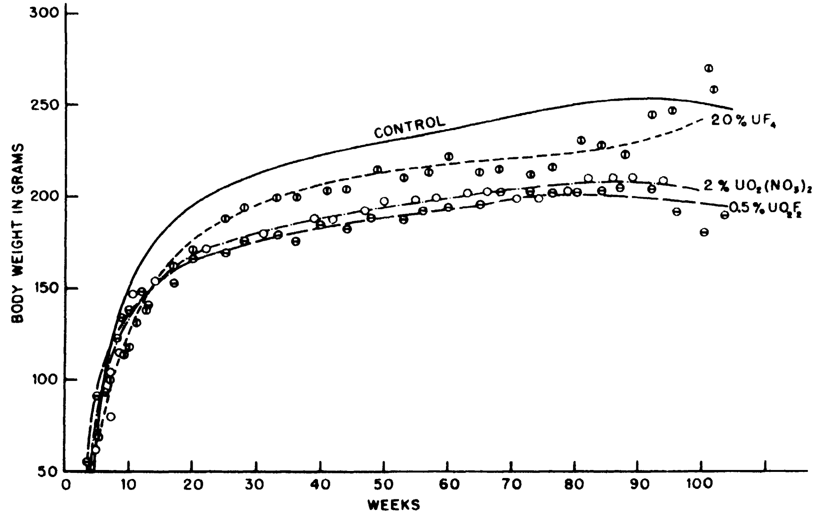

Fig. 7.22 —Growth curves for female rats fed certain uranium compounds.

somewhat greater than the reduction produced by 2 per cent uranyl nitrate hexahydrate, which, in turn, was greater than that produced by 20 per cent UF_4.

5.1 Body Weights. (a) Uranyl Fluoride. Male rats fed various levels of UO_2F_2 continued in the second year the tendencies that were established in the latter half of the first year (Tables 7.6 and 7.7). Amounts of 0.01 and 0.05 per cent showed no evidence of toxic reduction in body weight. One-tenth per cent in the diet gave weight reductions of 10 to 20 g in the second year that were of the same order of magnitude as observed on diets containing 0.15 per cent; 0.25 per cent in the diet gave weight depressions of 30 to 40 g; and 0.5 per cent gave weight depressions of around 100 g. In the females, 0.01 and 0.05 per cent in the diet produced the same body weights as those of the control rats; 0.25 per cent gave evidence of weight depressions of 30 to 40 g; and 0.5 per cent gave weight depressions of 50 to 80 g. One-tenth per cent in the diet was probably the lowest amount producing clearly detectable weight changes, and 0.05 per cent was probably the highest amount producing no effect on body weight.

(b) Uranyl Nitrate. During the second year the tendencies established in the latter half of the first year continued (Tables 7.9 and 7.10). Rats fed 0.01, 0.05, and 0.1 per cent uranyl nitrate hexahydrate in the diet showed no consistent tendency to differ in body weight from the average control rats; 0.5 per cent gave detectable weight depressions amounting to 10 to 25 g; 1.0 per cent gave weight depressions of 20 to 40 g; and 2 per cent gave weight depressions of 30 to 80 g. Five-tenths per cent in the diet seemed to be the amount that certainly gave toxic weight depressions, whereas 0.1 per cent was the maximum amount that failed to produce detectable change.

(c) Uranium Tetrafluoride. The tendencies established in the first year continued through the second year (Tables 7.11 and 7.12). The 0.5 per cent and 2.0 per cent UF_4 in the diets gave no evidence of a toxic depression of body weight. Both male and female rats fed 20 per cent showed consistently depressed body weights amounting to 15 to 35 g. No conclusions are drawn other than that the range of dose to produce detectable growth depression was placed somewhere between 2 and 20 per cent UF_4 in the diet.

(d) Uranium Dioxide. During the second year, there was no evidence that 0.5, 2.0, or 20 per cent UO_2 in the diet caused a weight depression (Tables 7.13 and 7.14). The UO_2 when fed to rats is practically nontoxic.

5.2 Mortality. During the second year the mortality increased steadily and rapidly (Table 7.8). The control rats, which had exhibited 12 per cent mortality in the males and 9 per cent in the females at the end of 12 months, showed 27 and 23 per cent mortalities, respectively, at 16 months; 46 and 52 per cent, respectively, at 20 months; and 64 and 61 per cent, respectively, at 24 months. As may be seen from the accompanying graph (Fig. 7.23), this increase in mortality followed a

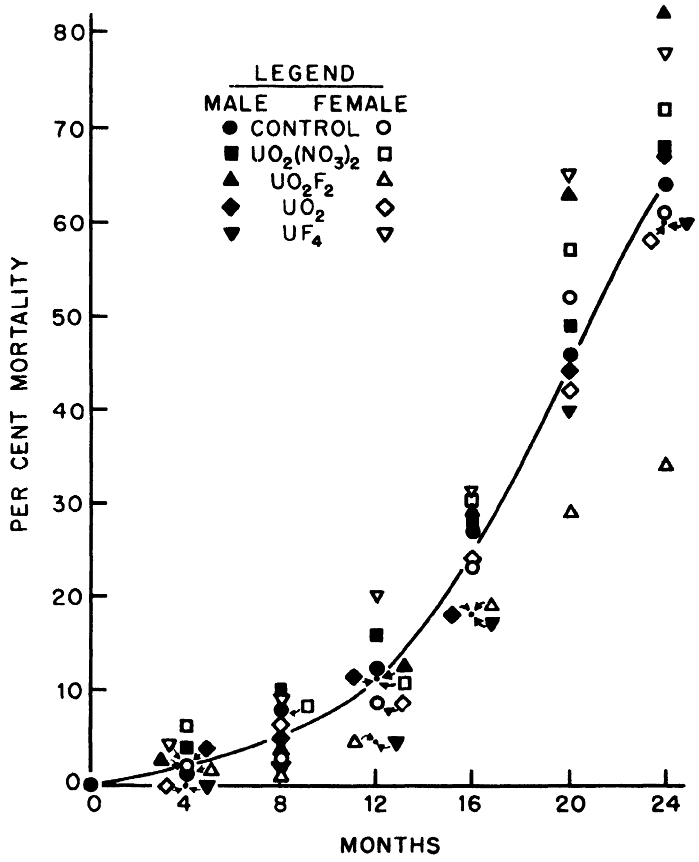

Fig. 7.23—Cumulative mortalities of rats fed various dietary levels of certain uranium compounds for 2 years.

smooth sigmoid curve. With the exception of the female rats fed diets containing up to and including 0.25 per cent UO_2F_2, all the experimental groups followed a pattern of increasing mortality closely parallel to that of the control rats. The group of female rats fed 0.25 per cent UO_2F_2 showed an aberrant low mortality at 20 and 24 months; no reason for this longevity is known. All the other rats, as is shown in

Table 7.8, exhibited mortalities between 60 and 82 per cent at the end of the second year. A few of the groups, e.g., the male rats fed UO_2F_2, showed consistently high mortalities during the second year. The females fed UF_4 also had a high death rate. Such a distribution, in which a highly toxic compound and also one of the least toxic are grouped together as producing among the highest death rates, emphasized the somewhat random character of the relationship between survival and toxicity of compound in the diet percentages given. There was no evidence that amounts of UO_2F_2 as great as 0.25 per cent, amounts of uranyl nitrate hexahydrate as great as 2 per cent, and amounts of UF_4 and of UO_2 as great as 20 per cent detrimentally influenced the life span.

5.3 Summary. 1. Four uranium compounds were fed to rats for 2 years, over which period of time the least amounts of the compounds that caused retardation of growth were determined as follows: UO_2F_2, 0.1 per cent; uranyl nitrate hexahydrate, 0.5 per cent; UF_4, 20.0 per cent; UO_2, 20 per cent, the highest level tested, produced no effect.

2. In general, the growth tendencies established by the rats in the latter part of the first year on the diet were continued throughout the second year.

3. The life span of rats was not affected by the ingestion of uranium compounds at dietary levels as high as here indicated: UO_2F_2, 0.25 per cent; uranyl nitrate hexahydrate, 2.0 per cent; UF_4 and UO_2, 20.0 per cent.

6. THIRTY-DAY FEEDING EXPERIMENTS, DOGS

A series of tests of exploratory nature was carried out on a minimum number of dogs to ascertain the approximate lethal level of oral intake. The dogs in our colony were mostly young beagles, weighing about 10 kg, and were healthy, lively animals. Each dog was placed in an individual cage, and the designated amount of a certain uranium compound was mixed in his diet. In some cases the diet became unpalatable and was refused. Such dogs were then given the material by capsule. The dogs were followed during a 30-day period in which the respective compounds were administered six times per week. In general, the program was to start with a dose that it was estimated the dog would tolerate, i.e., on which the dog would survive for the 30-day period. In the event that the dog succumbed prior to the end of the 30 days, another experiment was set up with a smaller dosage level; in the event that the dog survived, an increased dose was given a second dog. In this way it was possible to ascertain in rough limits a dose that was tolerated for 30 days.

Table 7.16—Mortality Data for Dogs Fed Various Uranium Compounds for 30 Days*

Compound	Dosage, g/kg body weight/day									
	20	10	5	2	0.5	0.1	0.02	0.01	0.005	0.001
UO_2F_2			Dead in 6 days		Dead in 4 days	Dead in 9 days	Dead in 11 days	Lived 30 days	Dead in 22 days	Lived 30 days
UCl_4			Dead in 4 days		Dead in 11 days	Dead in 22 days	Lived 30 days			
UO_4					Dead in 5 days		Lived 33 days			
$UO_2(NO_3)_2.6H_2O$		Dead in 6 days		Dead in 4 days	Dead in 10 days	Lived 30 days				
UO_3					Dead in 10 days	Lived 30 days				
$Na_2U_2O_7$					Dead in 10 days	Lived 30 days				
$(NH_4)_2U_2O_7$					Dead in 17 days	Lived 30 days				
High grade ore		Dead in 17 days	Dead in 10 days		Lived 30 days					
UF_4	Dead in 10 days	Dead in 19 days	Lived 30 days							
U_3O_8	Dead in 17 days	Lived 30 days								
UO_2	Lived 30 days		Lived 30 days							

*Heavily outlined entries represent the highest level of survival for each compound.

The mortality figures are given in the accompanying table (Table 7.16). The dosage is listed in grams per kilogram body weight per day. The most toxic compounds are given in the top of the table, the least toxic in the bottom of the table. It is apparent that the compounds can be divided roughly into three groups: those highly toxic, those moderately toxic, and those of slight toxicity.

In the first group are UO_2F_2, UCl_4, and UO_4; 0.01 g UO_2F_2/kg/day was tolerated; 0.02 g/kg/day of UCl_4 and of UO_4 was tolerated; 2 to 20 times these quantities was lethal.

In the moderately toxic group, 0.1 g each of uranyl nitrate hexahydrate, UO_3, $Na_2U_2O_7$, and $(NH_4)_2U_2O_7$/kg/day was tolerated, whereas five times this amount was lethal. With high-grade ore 0.5 g/kg/day was tolerated, whereas 10 times this quantity was lethal.

Table 7.17—Data on Various Criteria of Toxic Effects in Dogs Fed the Designated Uranium Compounds

Compound fed	Amount fed, g/kg/day	Days survived	Blood		Urine		Histopathology		
			NPN peak, mg %	Urea N peak, mg %	Sugar peak[a]	Protein peak, mg %[b]	Kidney injury[c]	Liver injury[d]	Gastro-intestinal hemorrhage[e]
None	0	30	34-37	11-13	0-+1		Normal	Normal	Normal
UO_2F_2	5	6	196	59	+1	1,050	+5	Abnormal fat	+3
	0.5	4	102	17	+3	750	N.S.[f]	N.S.[f]	+2
	0.1	9	472	400	+2	200	+4	Abnormal fat	+2
	0.02	11	375	188	+3	350	+3	Abnormal fat	+1
	0.01	30	52	17	+1	50	[g]		
UO_4	0.5	5			+3	550	+4	Degeneration, +1	+3
	0.02	30	55	48	+1	75	+1	Normal	Normal
UCl_4	5	4	67	28	+1	1,000	N.S.[f]	N.S.[f]	N.S.[f]
	0.5	11	336	185	+3	2,000	+4	Necrosis, +3	+3
	0.1	22	476	125	+2	450	+3	Normal	+2
	0.02	30	48	16	+1	30	+1	Normal	Normal
$UO_2(NO_3)_2 \cdot 6H_2O$	10	6	118	35	+2	1,500	N.S.[f]	N.S.[f]	+2
	2	4	234	77	+1	400	+4	Necrosis, +2	+3
	0.5	10	396	87	+2	1,200	+3	Abnormal fat	+1
	0.1	30	93	78	+2	100	+3	Normal	Normal
UO_3	0.5	10	?		+3	750	+5	Degeneration, +1	+3
	0.1	30	110	65	+4	550	+4	Normal	Normal
$Na_2U_2O_7$	0.5	10	51	14	+3	600	+4	Abnormal fat	Normal
	0.1	30	105	70	+2	475	+3	Normal	Normal

Table 7.17—(Continued)

Compound fed	Amount fed, g/kg/day	Days survived	Blood		Urine		Histopathology		
			NPN peak, mg %	Urea N peak, mg %	Sugar peak[a]	Protein peak, mg %[b]	Kidney injury[c]	Liver injury[d]	Gastro-intestinal hemorrhage[e]
$(NH_4)_2U_2O_7$	0.5	17	464	157	+3	1,500	+4	Normal	Normal
	0.1	30	210	136	+4	550	+3	Normal	Normal
U ore	10	17	508	200	+2	375	+5	Normal	Normal
	5	11	25	6	+2	140	+3	Degeneration, +1	+3
	0.5	30	176	100	+3	500	+4	Normal	Normal
UF_4	20	10	374	101	+2	1,000	+3	Abnormal fat	+1
	10	19	315	109	+2	80	+4	Normal	Normal
	5	30	100	55	+3	40	+2	Normal	Normal
U_3O_8	20	17			+5	1,050	+3	Degeneration, +2	Normal
	10	30	124	81	+3	120	+2	Normal	Normal
UO_2	20	30	142	102	+5	1,040	+3	Normal	Normal
	5	30	129	64	+4	1,000	+2	Normal	Normal

[a] Customary scale of 1+, 2+, etc., used with results of applying Benedict's reagent.

[b] Twenty-four hour samples not available.

[c] Injury to kidney is indicated on a comparative basis from mild (+1) to very severe (+5), regardless of the type of change. In general, the animals dying early showed a predominance of degeneration and necrosis; those surviving for 30 days showed a marked predominance of regenerative changes. See Chap. 4 for histopathological details.

[d] Liver injury is indicated on a comparative basis from mild (+1) to very severe (+5).

[e] Hemorrhage of the gastrointestinal tract is indicated on a comparative basis from mild (+1) to very severe (+3). See Chap. 4 for histopathological details. "Normal" signifies no hemorrhage.

[f] N.S., no section taken because of autolysis.

[g] This dog was on the diet for a 1-year period.

In the slightly toxic group, 5 g UF_4/kg/day was tolerated, whereas 10 g was not; 10 g U_3O_8/kg/day was tolerated, whereas 20 g was not; and 20 g UO_2/kg/day was tolerated.

A number of observations were made on each dog. The blood was examined at weekly intervals microscopically for amounts of NPN, urea nitrogen, and serum protein. The urine was examined for sugar, protein, pH, and specific gravity. Each dog was given a complete autopsy at the termination of the experiment. Table 7.17 shows data obtained from several of these tests. The compounds are listed in the same order as in Table 7.16. For each dog the number of days survived, the peak blood NPN, and peak urea nitrogen are given; the peak urinary sugar and protein are also included. Brief comments on the histological diagnoses of kidney, liver, and gastrointestinal tract injuries are included.

In examining the various criteria by which the effects of poisoning were followed in dogs, it is possible to select several that may be described as good, some that can be designated as of doubtful use, and some that apparently have no relationship to the changes occurring in uranium poisoning. The "good" criteria are blood NPN, urea nitrogen, urine protein, pathology, and sometimes urine sugar. Body

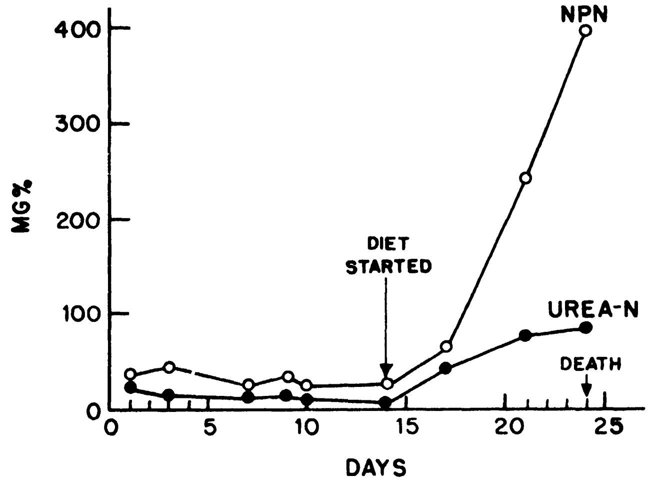

Fig. 7.24—Blood NPN and urea nitrogen curves for a dog fed 0.5 g of uranyl nitrate hexahydrate per kilogram of body weight daily.

weights, blood urea nitrogen figures, urinary specific gravity value, and 24-hr urine volume measurements are of doubtful value. The following measurements apparently have no simple relationship to the course of poisoning in dogs: serum protein, urinary pH, and microscopic observations of blood and urine.

Well-defined trends were evident in the high values of NPN and urea nitrogen of the blood. Typical increases in blood NPN and urea nitrogen in fatal poisonings were observed as illustrated in Fig. 7.24. Control values of NPN ranged from 25 to 42 mg %, and those of urea nitrogen ranged from 8 to 22 mg %. Four days after the dog was placed on a diet containing 0.5 g of uranyl nitrate hexahydrate per kilogram per day, these blood values began to increase, and on the 10th and final day were as follows: NPN, 395 mg %; urea nitrogen, 83 mg %. High levels of urinary sugar and protein were found in the severely injured animals that succumbed prior to the end of the experiment. It is interesting to observe that all the dogs exhibited a marked histological reaction in the kidney regardless of compound or dosage level. Thus it is clearly established that an animal can survive a 30-day period with a severe kidney injury.

Attention should be called to the fact that a number of these animals died without a marked increase in blood NPN. Particularly was this phenomenon observed in dogs that died in the first few days on test. This condition resulted from more than one compound, e.g., when UO_2F_2, UCl_4, and uranyl nitrate were fed, the dogs that died in 4 to 6 days showed NPN values varying from less than 100 to approximately 200 mg %. In contrast, the dogs that died in 9 to 22 days showed values of 300 to 500 mg %. It seemed that death had occurred before the NPN had time to reach maximum values.

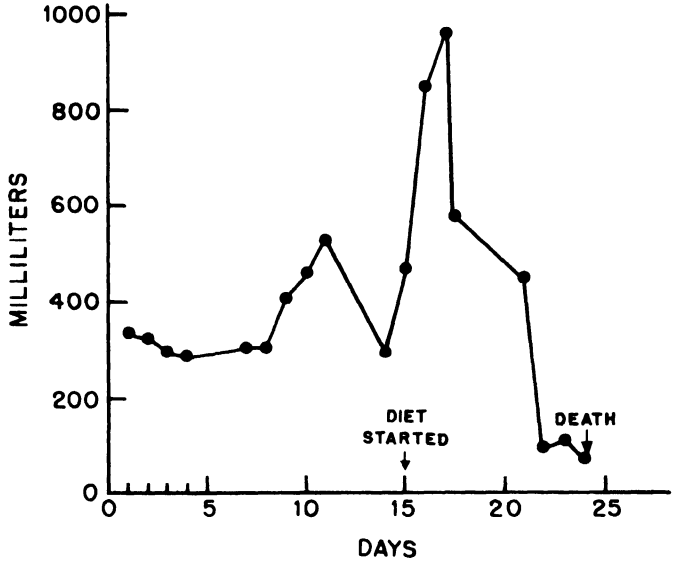

Fig. 7.25—Twenty-four-hour urine-volume curve for a dog fed 0.1 g UO_2F_2/kg of body weight daily.

Glycosuria and proteinuria were constant concomitants of the poisoning. It was the glycosuria that led physicians years ago to treat diabetes mellitus by administering uranium salts on the old homeopathic theory that a drug which produced a symptom should be used to treat the symptom.

The lack of effect on the hematological picture is outstanding. Even in those animals so severely poisoned that they died in 1 or 2 weeks there were no changes in red-cell count, percentage of hemoglobin, or differential white count sufficient to be designated as abnormal.

The urinary protein excretion reached extremely high levels in the animals most severely poisoned, especially in three that died in the first few days after starting the diets. This might be described as a nephrotic type of kidney injury in which the kidney is rendered porous to the blood proteins. The urinary volume was frequently observed to follow a course of polyuria immediately succeeded by oliguria. This sequence is typical and informative; unfortunately, the difficulties of quantitative urine collections were so great that good data were only occasionally obtained. A striking illustration of the progress of kidney injury as shown by the urine output is given in Fig. 7.25. This dog excreted 290 to 525 ml of urine per day in a 2-week control period. On the day following the administration of 0.1 g UO_2F_2/kg of body weight the urinary excretion rose to 850 ml; on the next day, 960 ml was excreted. Then the oliguric phase began, and excretions fell to 580, 450, 100, 115, and 75 ml on succeeding days.

6.1 Pathological Studies of Liver and Gastrointestinal Tract. Consistent abnormalities of the livers, gastrointestinal tracts, and oral mucosae were found in many of the dogs (Table 7.17). Dogs ingesting UO_2, U_3O_8, and UF_4 showed normal gastrointestinal tracts, except the animal receiving the highest dose of UF_4 (20 g/kg/day), in which animal a slight hemorrhage was seen. In these dogs the livers appeared normal except in the animals receiving U_3O_8 and UF_4 at a rate of 20 g/kg/day. At these dosages moderate degeneration and excess fat were observed. With the other compounds there was a consistent finding of normal gastrointestinal tracts and normal livers in the surviving animals, but varying degrees of gastrointestinal hemorrhage and liver damage in dogs fed the higher dosage levels. Ulcers of the oral mucosa were observed in animals fed UCl_4 (all levels), high-grade ore (5 g/kg/day), UF_4 (20 g/kg/day), UO_3, and $Na_2U_2O_7$ (0.5 g/kg/day only). There is no evidence that the liver changes were primary responses to uranium poisoning. In fact, until evidence to the contrary is established, it may be that the liver changes were secondary to decreased liver function. It should be emphasized that the dosages producing these patent injuries were high doses.

6.2 Comparison of Toxicity, Dogs vs. Rats. It is possible to compare the toxicity for these various compounds in the rat and in the dog when the compounds are administered orally. In Table 7.18 the compounds are in three groups: those most toxic, those of intermediate toxicity, and those least toxic. In this table the grams per kilogram per day are given for each compound as (1) the smallest estimated amount producing a weight loss in weanling rats during a 30-day feeding period, and (2) the highest amount ingested daily by a dog that survived for 30 days. The value for the rat is computed on the assumption that the average weanling rat ingests 10 g of food per day. This is probably a low figure, since our weanling rats have been known to ingest 13 to 15 g on the average.

Table 7.18 — Comparison of Toxicity of Various Uranium Compounds in Dogs and Rats

Compound	Rat, smallest amount producing wt. loss in 30 days, g/kg/day	Dog, highest amount ingested daily with survival for 30 days, g/kg/day	Comment
UO_2F_2	0.05	0.01	Most toxic
UO_4	0.05	0.02	Most toxic
UCl_4		0.02	Most toxic
UCl_4	0.25		Mid-toxic
$UO_2(NO_3)_2.6H_2O$	0.25	0.1	Mid-toxic
UO_3	0.25	0.1	Mid-toxic
UO_2Ac_2	0.25		Mid-toxic
$Na_2U_2O_7$		0.1	Mid-toxic
$(NH_4)_2U_2O_7$		0.1	Mid-toxic
High-grade ore		0.5	Mid-toxic
UF_4	10	5	Least toxic
U_3O_8	10	10	Least toxic
UO_2	10	20	Least toxic

It must be emphasized that, for rats, these values are the minimum dietary levels producing detectable injury as shown by body weight. On the contrary, for the dog these values represent something like the maximum dietary level that can be taken for 30 days. It is impossible to say what fraction of the tolerated dose in the dog would be the minimum to produce a detectable sign of injury in some well-established criterion such as NPN or proteinuria. It may require only one-tenth, or perhaps less, of the amount stated in the table to produce these minimal effects. Consequently the table as it stands gives a comparison that shows the dog to be relatively more resistant than it actually is. In glancing down the table, a factor of 2 to 5 is frequently found between the dose for rats and the dose for dogs, i.e., one-half to one-fifth of the dose giving minimal signs in the rat has been successfully ingested for 1 month by a dog. The relative resistance of the two species would probably be better represented by at least a tenfold increase in this ratio of susceptibilities. Thus, 10 to 50 times the dosage that would produce detectable injury following ingestion by the dog would probably be required to give evidence of injury in the rat.

6.3 Summary. 1. A series of tests of exploratory nature were performed on a minimum number of dogs to ascertain the approximate lethal level of oral intake. Single dogs were fed a certain daily dose of one of the compounds under investigation. Within rough limits the daily dose that could be ingested with survival for 30 days was established.

2. A considerable number of criteria of uranium poisoning in dogs were observed in 30-day feeding experiments. The following criteria proved the most useful: survival for 30 days, blood NPN, urea nitrogen, and the histopathology of the kidney, liver, and gastrointestinal tract.

3. Using various criteria as a basis for determining the relative toxicities of the several uranium compounds, the following classification was made:

Slightly toxic	Moderately toxic	Highly toxic
UF_4	Uranyl nitrate	UO_2F_2
U_3O_8	UO_3	UCl_4
UO_2	$Na_2U_2O_7$	UO_4
	$(NH_4)_2U_2O_7$	
	High-grade ore	

4. Dogs were more susceptible to uranium poisoning than rats.

7. ONE-YEAR FEEDING EXPERIMENTS, DOGS

From the results of the 30-day studies, several dietary levels of UO_2F_2, UCl_4, and uranyl nitrate were selected, and a few dogs were fed these diets for a period of 1 year. Each dog was placed in an individual cage. In general, a regimen similar to that of the 30-day study was maintained before adding the toxic materials to the diets. All the dogs were studied for a conditioning period of 5 weeks to accustom them to the routine of the experiment. In this procedure a series of values was obtained on each dog, which served as the control values for that dog in addition to the control values obtained on a separate group of dogs fed the regular stock ration throughout the year. During this preliminary period and during the first month on the experimental diets, blood samples were taken at weekly intervals for estimations of NPN and urea nitrogen, for serum protein, and for hematological studies. Urine samples were also collected weekly for sugar and protein measurements, for specific gravity, and for pH determinations. Twenty-four-hour volume measurements were recorded. The dogs were observed daily for clinical condition, appetite, condition of feces, etc. (see Table 7.19). To provide a proper normal base line by which to assess the degree of injury, a group of 14 control dogs was studied at the same time on the same plan.

Table 7.19—Data on Dogs Fed Various Uranium Compounds for a Period of 1 Year

Compound	G/kg/day	Time survived	Blood NPN peak, mg %	Blood urea N peak, mg %	Urine sugar peak	Urine protein peak, mg %
None			34-77	11-13	0 to +1	
UO_2F_2	0.01	1 year	52	19.2	+1	50
	0.0025	1 year	64	16	0	35
	0.0025	1 year	54	20	+1	15
	0.001	1 year	65	15	0	70
	0.001	1 year	76	15.4	0	5
	0.0002	1 year	67	19.2	+1	25
	0.0002	1 year	48	19.6	+1	125
UCl_4	0.05	1 year	81	25.8	+4	650
	0.05	1 year	51	20	+1	100
	0.01	1 year	56	19.2	+1	55
	0.01	1 year	58	16.4	+1	35
	0.002	1 year	51	19.6	+1	65
	0.002	1 year	67	17.9	+1	20
$UO_2(NO_3)_2.6H_2O$	0.2	138 days	109	63	+2	325
	0.2	1 year	148	113	+2	125
	0.1	1 year	102	65	+2	375
	0.1	1 year	80	19	+2	300
	0.02	1 year	67	21.8	+2	400
	0.02	1 year	76	21	+3	600

With the exception of one of the dogs on the highest level of uranyl nitrate, all the dogs survived the 1-year experiment. In general, their appetites were good, they appeared healthy and active, and for the most part showed no effect of the incorporation of various uranium compounds in their diets. No extreme elevations of blood NPN or urea nitrogen were observed. Most of the dogs exhibited some urinary sugar and some evidence of proteinuria during the year. At this writing the results of histological examinations or hematological studies are not available.

7.1 Uranyl Fluoride. The selection of levels for the number of grams of UO_2F_2 per kilogram per day was influenced by the unfortunate coincidence of a selection in the 30-day studies of two dogs that were apparently highly susceptible to UO_2F_2. Since one dog receiving 0.005 g/kg/day had died in 22 days, dietary levels were chosen below 0.005 g/kg/day. Thus two dogs were given doses of 0.0002, 0.001, and 0.0025 g/kg/day each for 1 year. During the year the dogs showed no apparent effects of the inclusion of such small amounts of UO_2F_2 in the diet. As may be seen in the table, only traces of sugar were found in the urine, and insignificantly small amounts of protein appeared in the urine; the blood NPN and urea nitrogen values were all within the normal range. Late in the year when facilities became available, an additional dog was placed on a diet containing 0.01 g UO_2F_2/kg/day. This dog also failed to show any signs of a toxic response.

7.2 Uranium Tetrachloride. Two dogs were each fed the following amounts of UCl_4: 0.002, 0.01, 0.05 g/kg/day, respectively, for a period of 1 year. The two dogs receiving 0.05 g/kg/day showed some effects of the toxic material in the early part of the experiment. This consisted of a loss of appetite, lassitude, and some loss in weight, which persisted for only a few days. Recovery was complete, in so far as could be judged from the appearance and activity of the dogs, and the 1-year feeding period was successfully continued. Some evidence of a toxic effect is seen in Table 7.19. One of these dogs exhibited a peak blood NPN value of 81 mg %, considerable sugar appeared in the urine, and the proteinuria reached 650 mg %.* About a month after this episode the other dog receiving 0.05 g/kg/day showed clinical signs of illness without the accompanying biochemical manifestations. It was at this time that the highest values recorded for the second dog were obtained. The dogs receiving 0.01 and 0.002 g/kg/day gave only normal findings throughout the year.

7.3 Uranyl Nitrate. Of six dogs fed uranyl nitrate hexahydrate, two received 0.02, two received 0.1, and two received 0.2 g/kg/day. One of the dogs receiving the largest dietary addition died after 138 days on the diet. This death came at the peak of one of the cyclical injury states referred to previously. The dog showed elevated blood NPN and urea nitrogen values. Glycosuria and proteinuria accompanied the exacerbation of toxic symptoms. The other dog receiving the same dosage exhibited a concurrent picture of illness and showed even more highly elevated blood NPN and urea nitrogen values. However, this dog survived. All the other dogs showed no clinical signs of poisoning. However, more than traces of sugar were occasionally found in the urines, and, at one time or another, substantial amounts of urinary protein appeared. Peak blood NPN values were observed which were double or more than double the control level in each of these dogs; however, no special significance is attached to these changes.

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* Twenty-four-hour samples were not available, and throughout this work the concentration of urine constituents is expressed in terms of milligram per cent.

Perhaps these findings may be summarized by saying that amounts of 0.02 g/kg/day, or greater, of any one of the soluble uranium compounds were capable of producing evidences of poisoning in some one of the criteria employed. Body weights may not have changed, the appetite may have remained normal, obvious signs of illness may not have appeared, but indications of injury may have been found in an elevated blood NPN, a heightened urinary protein excretion, or histological changes in the kidney.

7.4 Uranium Dioxide. Of six dogs fed uranium dioxide, two received 1, two received 5, and two received 10 g/kg/day. At the end of 2 months of the planned 1-year experimental period, all six dogs appeared to be in good health.

7.5 Uranium Tetrafluoride. Of six dogs fed uranium tetrafluoride, two received 0.2, two received 1, and two received 5 g/kg/day. At the end of about 2 months of the planned 1-year experimental period, all six dogs appeared to be in good health.

7.6 Body Weights. The dogs were weighed at weekly intervals throughout the year. The initial weights and the average percentage changes in body weight at 3, 6, 9, and 12 months are given in Table 7.20.

Of the seven dogs fed various amounts of UO_2F_2 as indicated, only one lost weight. The others all showed gains of 10 to 40 per cent. The one that lost weight received the highest level, namely, 0.01 g/kg/day.

Of the six dogs fed various amounts of uranyl nitrate hexahydrate as indicated, the two receiving the highest amount, i.e., 0.2 g/kg/day, showed severe losses in weight, and one of these dogs failed to survive. The other four dogs showed gains in weight of 1 to 40 per cent.

All the six dogs fed various amounts of UCl_4, as indicated, gained 10 to 40 per cent in body weight during the year.

All the 14 control dogs gained weight, which on the average amounted to 18 per cent during the year.

It is possible to show an over-all relationship of daily dose to body weight (see Table 7.21). It appeared that a daily dose of uranyl nitrate hexahydrate of the order of 0.2 g/kg/day will produce a loss in body weight in the adult dog, whereas 0.1 g/kg/day will not. It should be

Table 7.20 — Body-weight Changes in Dogs Fed Various Uranium Compounds for 1 Year

Compound	Amount fed, g/kg/day	Initial wt., kg	Body-weight change, %			
			3 mo.	6 mo.	9 mo.	12 mo.
UO_2F_2	0.01	6.6	−4.5	−6	−1.5	
	0.0025	8.7	5.75	21.8	36.8	39
	0.0025	8.4	8.3	31	29.7	26
	0.001	7.4	6.75	25.6	31	35
	0.001	9.1	2	4	16.4	17.5
	0.0002	7.7	5.2	14	32	40
	0.0002	5.1	17.6	9.8	19.6	11.8
$UO_2(NO_3)_2$ $.6H_2O$	0.2	7.3	−9.5	−35.6		
	0.2	9.3	−10.7	−25.8	−15	−32
	0.1	10.5	−4.7	3.8	12.4	14.3
	0.1	6.6	6	32	38	33
	0.02	8.4	4.75	8.3	3.6	1
	0.02	10.5	−1	4	3	11.5
UCl_4	0.05	6.5	15.4	38.5	29	23
	0.05	9.1	5.4	29.6	32	35
	0.01	8.0	7.5	27.5	30	36
	0.01	11.4	4.4	3.5	7	11.4
	0.002	10.2	−14.7	0	15.7	17.6
	0.002	8.4	0	8.3	14.3	16.7
Controls*	None	7.4	3.24	6.62	12.43	18.24

*Average of 14 values.

Table 7.21 — Effect of Various Uranium Compounds on the Body Weight of Dogs Fed Daily Doses for 1 Year

Amount fed, g/kg/day	Compound	Change in body wt.
0.2	$UO_2(NO_3)_2$	Loss
0.1	$UO_2(NO_3)_2.6H_2O$	Gain
0.05	UCl_4	Gain
0.02	$UO_2(NO_3)_2$	Gain or ±
0.01	UO_2F_2	±
0.01	UCl_4	Gain
0.002	UO_2F_2	Gain
0.002	UCl_4	Gain
0.001	UO_2F_2	Gain
0.0002	UO_2F_2	Gain
None		Gain

noted that these results apply only to dogs fed uranyl nitrate hexahydrate; doses of UO_2F_2 or UCl_4 were not so large. There was no proof that ingestion of 0.1 g/kg/day of these compounds might not also have produced weight loss.

7.7 Blood NPN. In some of the dogs the blood NPN values followed a series of changes sufficiently characteristic to raise the question of a pattern of cyclical injury. For example, in the case of one of the dogs fed uranyl nitrate hexahydrate, 0.02 g/kg/day, the blood NPN rose promptly in the second week following the establishment of the experimental diet, from normal levels of about 30 mg % to a peak of 50 mg % (see Fig. 7.26). Values not far from the normal range were thereafter recorded for a period of about 14 weeks, whereupon a second marked peak occurred with the high value of 67 mg % in the 24th week. In the 30th to 38th weeks, NPN values were again much lower (37 to 39 mg %). However, a tendency toward increasing values is again observed toward the end of the year.

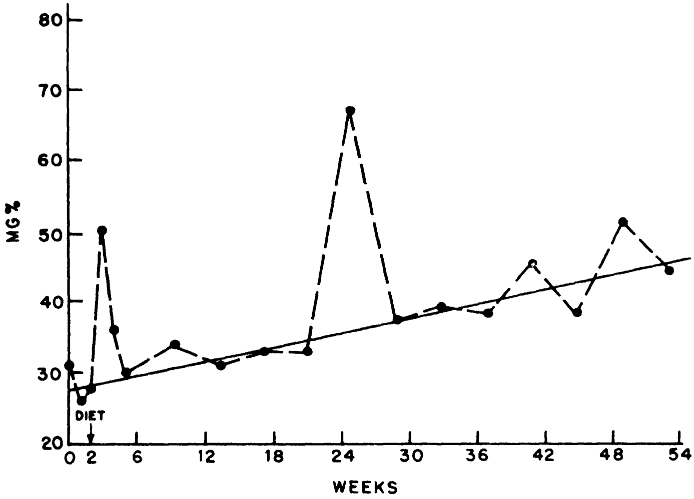

Fig. 7.26 — Blood NPN curve for a dog fed 0.02 g of uranyl nitrate hexahydrate per kilogram of body weight per day.

One of the most impressive facts about the values illustrated in Fig. 7.26 is the inclination of the base line of NPN values. These might be interpreted as evidence of a continued and increasingly potent injury state. The question that this pattern raises is as follows: Is there a recurrent peak of injury that occurs at the 20th week or a little later, and at the 50th week or a little later? This recurrent injury superimposed on the evidence of a steadily increasing azotemia may eventually produce serious intoxication in a dog that has survived for a period of 1 year.

7.8 Blood NPN and Body-weight Changes. An interesting example of the periodicity of blood NPN changes as related to body-weight changes is shown in Fig. 7.27. Here the blood NPN values and the body weights of the two dogs fed 0.2 g of uranyl nitrate hexahydrate per kilogram per day are plotted against the biweekly periods for the 1-year experiment.

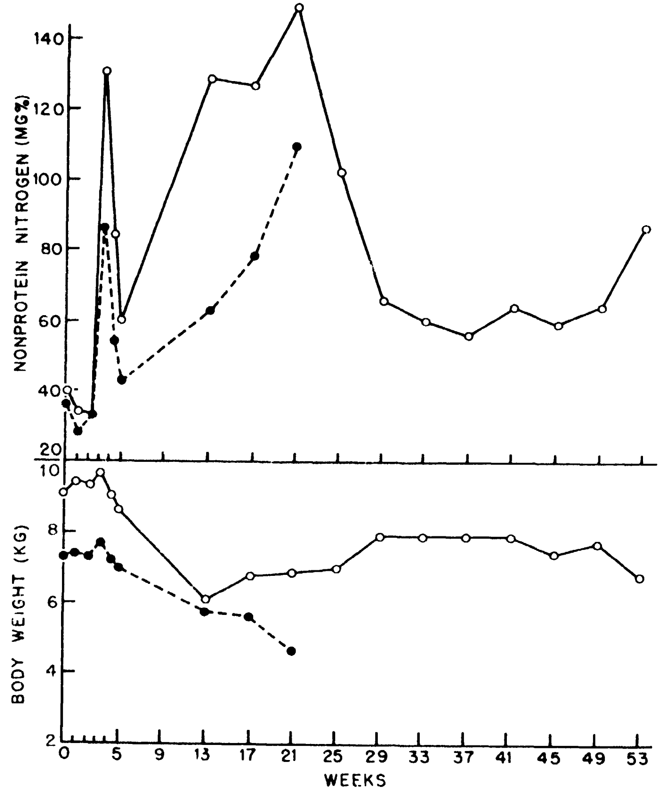

Fig. 7.27 —Nonprotein nitrogen curves (above) and growth curves (below) for two dogs fed 0.2 g of uranyl nitrate hexahydrate per kilogram of body weight daily.

For the dog that survived, a value of 130 mg % was recorded—the initial NPN peak value that occurred in the second week after the experimental diet was started. This value was followed by one much lower (60 mg %), which was still considerably above normal range. High values were then found, which reached their peak a little later than the 20th week. The trend thereafter was toward values of the order of 60 mg %, which persisted until the 52nd to 54th weeks, when a tendency toward another peak was observed. The close resemblance of these changes in time to those of the dog illustrated in Fig. 7.26 should be noted.

The body weight of the surviving dog (Fig. 7.27) fell markedly at the time that the first peak in blood NPN appeared. The weight fall continued during the period of the second major peak centering around the 20th week. During the latter part of this episode, the body weight increased somewhat and leveled off until the 48th to 50th weeks, but remained at a value some 20 per cent less than the original body weight. During the last weeks of the experiment when the blood NPN tended to rise, the body weight again fell steadily. Apparently there was a reciprocal relationship during the course of the experiment in this way: When recurrent episodes of poisoning as indicated by peaks of NPN in the blood occurred, there were decreases in body weights at the same time intervals. Both of these changes can be interpreted as evidences of uranium poisoning.

The dog that died (Fig. 7.27) gave evidence of the initial uranium injury by a blood NPN peak value of 86 mg % in the second week following the initiation of the experimental regimen simultaneously with the NPN peak in the surviving dog. Later the values returned almost to normal (43 mg %), and then began a steady and marked increase that terminated in the death of the animal in the 21st week. Again this second peak of blood NPN values paralleled the values found in the surviving dog. The closely parallel losses in body weights in the two dogs is plainly shown in Fig. 7.27. The dog that died began to lose weight at the time of the first peak in blood NPN. The body weight of this animal, unlike that of the surviving dog, continued an almost linear decrease during the second episode of increasing blood NPN values and reached a low value of nearly 40 per cent less than the initial body weight at the time of death.

The facts to be stressed by a consideration of this experiment are (1) evidence of recurrent cycles of injury when uranium compounds are fed to dogs and (2) the opposing simultaneous trends of blood NPN increase and body-weight decrease in uranium poisoning.

It should be emphasized that these two dogs received the largest amount of uranium administered in any of the 1-year experiments. Body-weight changes were not seen in dogs fed lesser amounts (see Table 7.21).

7.9 Comparison of Toxicity, Dogs vs. Rats. Some idea of the relative susceptibilities to three compounds are given in the accompanying Table 7.22. Here the highest dietary levels tolerated chronically by rats and dogs are indicated. The feeding experiments for rats were carried out for 2 years, and the feeding experiments for dogs for 1 year, except in the case of UF_4, on which information for only the first 10 weeks is available. When UO_2F_2 is fed, rats tolerate 0.25 g/kg/day without increase in mortality but with a severe depression in body weight. A dog given 0.01 g/kg/day showed signs of toxic effects during the 1-year period but survived. For this compound, rats appear to be more resistant by a factor of about 25. When uranyl nitrate hexahydrate is fed, rats tolerate 2 g/kg/day without increased mortality by the end of the 2-year period. Dogs fed 0.1 g/kg/day did not exhibit weight loss and survived, whereas dogs fed 0.2 g/kg/day exhibited weight loss and one of two dogs died. Rats are more resistant to ingestion of uranyl nitrate by a factor of about 20.

Table 7.22—Highest Dietary Level Tolerated Chronically

	Amount, g/kg/day	
	Rats (2 years)	Dogs (1 year)
UO_2F_2	0.25	0.01
$UO_2(NO_3)_2.6H_2O$	2.0	0.1
UF_4	20.0	5.0*

*First 10 weeks.

The situation is somewhat different in the case of the insoluble, relatively nontoxic UF_4. When 20 per cent of this material was included in the diet of rats, some weight reduction was observed, but it is improbable that this is the highest dietary level that might be tolerated chronically. Dogs fed 5 g/kg/day showed signs of poisoning; however, they survived the year period. These data give a factor of 4 in favor of the rats. Thus, on the basis of this criterion, the dog was found to be 5 to 25 times as susceptible to uranium poisoning as the rat.

7.10 Summary. 1. A few dogs were fed various levels of UO_2F_2, UCl_4, and uranyl nitrate for 1 year. Additional tests of similar plan have been conducted in which UO_2 and UF_4 were fed.

2. Body-weight losses were observed only in the two dogs fed the highest level of uranyl nitrate. No body-weight losses were observed at any of the other levels of any of the other compounds.

3. A number of criteria of uranium poisoning were observed. Evidences of poisoning were found in some of these criteria when amounts greater than 0.02 g/kg/day were added to the diets.

4. A question of the existence of a cyclical pattern of chronic uranium injury has been raised, which requires further experimentation to establish.

8. THIRTY-DAY FEEDING EXPERIMENTS, RABBITS

A single 30-day feeding experiment was conducted in which uranyl nitrate hexahydrate was administered orally to rabbits at levels of 0, 0.02, 0.1, and 0.5 per cent for a period of 30 days. Body weights were recorded twice weekly, clinical observations were made, and a gross pathological study was conducted at the termination of the experiment. Kidneys only were examined histologically.

8.1 Body Weights. In Fig. 7.28 body weights are expressed as percentages of the initial weights of the rabbits. It may be seen that at a level of 0.5 per cent the rabbits lost weight rapidly until death ensued by the 10th day. At a dietary level of 0.1 per cent the average body weight fell to a minimum of 80 per cent of the initial weight on the 14th day and remained at approximately this level through the 21st day. During the period of depressed growth, four of the six rabbits died. During the last 10 days of the experiment, the two surviving animals regained their initial weights. The rabbits that received 0.02 per cent uranyl nitrate hexahydrate attained 108 per cent of their initial weight on the 4th day and then dropped to an average of 101 per cent on the 7th day. The same weight level was retained through the 10th day, after which a steady increase in average body weight was noted through the 27th day of the experiment. A second period of declining body weight occurred during the last 4 days on the experimental diet.

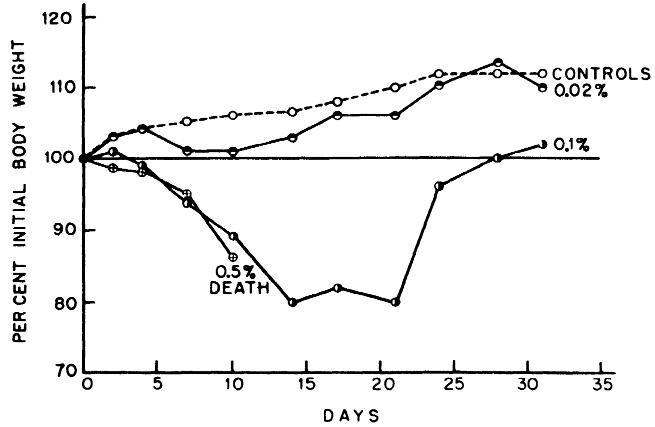

Fig. 7.28 —Body-weight changes in rabbits fed various dietary levels of uranyl nitrate hexahydrate.

8.2 Mortality. At a dietary level of 0.5 per cent uranyl nitrate hexahydrate, the rabbits all died between the 7th and 12th days (Fig. 7.29). The first mortality at the 0.1 per cent level occurred on the 11th day, and the fourth and final death occurred on the 23rd day. No rabbits fed 0.02 per cent uranyl nitrate hexahydrate died in the 30-day experimental period.

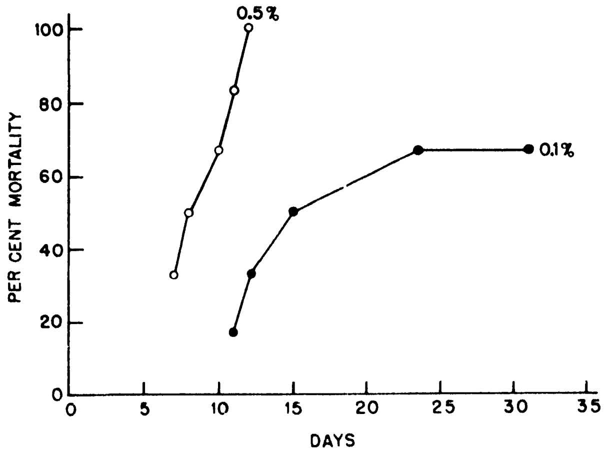

Fig. 7.29 —Mortality curves for groups of rabbits (six per group) fed various percentages of uranyl nitrate hexahydrate in the diet.

8.3 Pathology. At the time of sacrifice, no gross changes traceable to uranium poisoning were noted. When the kidneys were examined histologically, renal damage was observed at all dietary levels, being moderate at the two lower levels and moderately severe at the highest level.

8.4 Relative Susceptibilities of Rabbits, Dogs, and Rats. The somewhat limited data available on these three species nevertheless permit two comparisons of susceptibilities to uranium poisoning. The more striking comparison is on the basis of amounts ingested daily (strictly speaking, the amounts as percentages of uranium compounds in the diets on the assumption of normal food ingestion) during 30-day feeding experiments. When the mortalities observed on various diets are plotted as ordinates against the logarithm of the dietary levels in grams per kilogram per day as the abscissas (Fig. 7.30), three clearly separated lines are obtained. The rabbit is much more susceptible than the dog, which in turn is much more susceptible than the rat.

Interestingly enough, it happens that there is a factor of nearly 10 between each line. When the LL_{50}'s (lethal level to give a 50 per cent mortality) are interpolated from the meager data available, the following values are obtained: LL_{50} for rabbits is of the order of 0.04 g/kg/day, for dogs of the order of 0.1 g/kg/day, and for rats of the order of 4 g/kg/day. These numbers are probably highly inaccurate, but they represent approximately the magnitude of the difference in susceptibility of the three species.

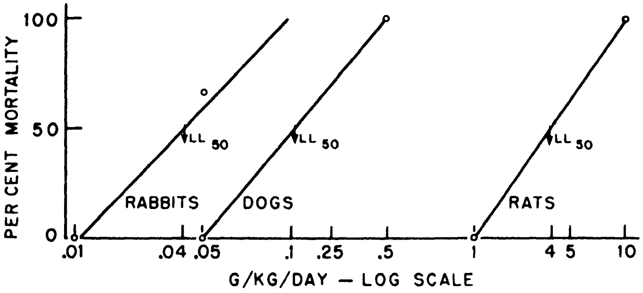

Fig. 7.30—Mortality in 30-day feeding tests with uranyl nitrate hexahydrate.

The second manner in which a comparison of the susceptibility of these three species may be made is in the minimal amount of uranyl nitrate in the diet that will produce a detectable weight depression. As indicated below, in the young adult rabbit 0.01 g/kg/day will give a detectable weight depression. In the dog, between 0.1 and 0.2 g/kg/day, or 10 to 20 times the amount producing a similar change in the rabbit was required. Thus by this criterion the same order of susceptibility is established; the rabbit is more susceptible than the dog, which in turn is more susceptible than the rat. It should be noted that again a factor of nearly 10 is involved in the differences in each case.

Minimal Amount of Uranyl Nitrate Hexahydrate in the Diet to Cause Detectable Weight Depression

Rabbit	Dog	Rat
0.01 g/kg/day	0.1–0.2 g/kg/day	0.5 g/kg/day

8.5 Summary. 1. Small groups of rabbits were fed various levels of uranyl nitrate in the diets.

2. At each level, body-weight losses were recorded; these were minimal in the rabbits receiving 0.02 per cent.

3. All the rabbits receiving 0.5 per cent uranyl nitrate hexahydrate died; none of the rabbits receiving 0.02 per cent in the diet died.

4. Histological renal damage was observed at all dietary levels.

Chapter 8

THE TOXICOLOGY OF COMPOUNDS OF URANIUM FOLLOWING APPLICATION TO THE SKIN

By James A. Orcutt*

The possible occurrence of poisoning from dermal exposure of industrial workers to salts of uranium, especially ethereal solutions of uranyl nitrate, has made it desirable to secure experimental information regarding this subject. In addition it is of importance to be able to evaluate the degree of inadvertent percutaneous intoxication during toxicological experiments involving other routes of administration, such as pulmonary exposure to uranium-containing dusts. Heretofore little has been reported concerning the toxicity resulting from the absorption of uranium compounds through the skin.

DeLaet and Meurice[1] studied four workers who in spite of protective precautions were known to have been subjected to dermal and pulmonary exposure. Of these, two were exposed to the soluble uranyl nitrate and two to the insoluble sodium diuranate for periods of from 7 to 24 months. Samples of urine were tested for pH, density, organic material, inorganic material, total dissolved material, water, sulfuric acid, oxalic acid, urea, uric acid, phosphoric acid, chlorides, glucose, albumin, and bile pigments. Little was found to be abnormal. Evaluation of this report was difficult because the relative amounts of uranium absorbed by the skin and by the lungs were unknown.

Jaffe[2] reported that workers who had been subjected to daily dermal exposures of uranyl nitrate had "insignificant" amounts (of the order of 0.01 mg of uranium per liter) in their urines. No clinical abnormalities seen in these workers were considered attributable to uranium salts.

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In the present investigations, rabbits, guinea pigs, rats, and mice were used as experimental animals. Whenever possible, animals of the same age, sex, and strain were employed for each experiment unless it was possible to show that any one of these variables did not influence the experimental results. When groups of animals of widely divergent weight ranges were used, they were first separated into groups according to weight. They were then distributed so that each dose group represented a cross section of the entire range of weights. This was done so that the possible susceptibility of any particular weight group would not bias the values obtained at any portion of the dosage-mortality curve.

The rabbits, which were adult males and females of New Zealand White, New Zealand Red, Checker, and Chinchilla strains, as well as mixed breeds, ranged in weight from 2 to 4 kg.

The guinea pigs, males and females of various colors and ages, ranged in weight from 250 to 750 g. All, however, were growing animals.

The rats, young females of the Wistar strain, ranged in weight, with few exceptions, from 100 to 150 g.

The mice were young female albinos, of various strains, most of which weighed from 16 to 21 g.

1. ACUTE POISONING

1.1 Mortality. Before initiating experiments for the purpose of collecting mortality data, it was necessary to establish a suitable range of doses to be used for each species. To this end, preliminary, or pilot, experiments, involving small groups of animals exposed to various single doses of uranyl nitrate hexahydrate, $UO_2(NO_3)_2.6H_2O$, were performed for each species. From the results thus obtained, a range of graded doses, estimated as potentially lethal for from 20 to 80 per cent of a given species, was chosen for use in subsequent experiments with larger groups of animals. These estimated ranges were corrected, when necessary, in the light of additional data. Quantitative statistical studies of the relationships between dosage and mortality were made for uranyl nitrate hexahydrate only.

(a) Uranyl Nitrate Hexahydrate. Information was obtained that industry employed an ethereal solution compounded by mixing approximately equal parts by weight of ether and uranyl nitrate hexahydrate. In an attempt to simulate industrial conditions, therefore, the animals were exposed to a solution compounded in the same manner.

Since the specific gravity of ether is 0.71 at 25°C, 7.1 g of uranyl nitrate hexahydrate was added to each 10 ml of ether. There resulted a biphasic system in which uranyl nitrate was distributed between the ethereal layer and an aqueous layer formed from the water of crystallization. The aqueous layer was discarded.

Although the original form of the compound was the crystalline $UO_2(NO_3)_2.6H_2O$, a large part of the water of crystallization was lost when the solution in ether was made. The recrystallization products on the skin, after the ether had evaporated, probably contained the anhydrous uranyl nitrate, the dihydrate, the hexahydrate, and uranyl nitrate plus ether of crystallization. It is not known which of these forms of the compound actually penetrated the skin. All dosages of the compound, therefore, have been calculated, arbitrarily, on the basis of the original form before solution, i.e., the hexahydrate, and in addition have been listed in terms of elemental uranium.

In order to determine the content of uranium in the ethereal layer, aliquots were weighed and evaporated to dryness at 70°C. From the weight of the residue (assumed to be anhydrous uranyl nitrate) a 71 per cent concentration of uranium, as $UO_2(NO_3)_2.6H_2O$, was calculated. Photocolorimetric determinations made, using the uranium ferrocyanide complex, by Cohenour and fluorophotometric determinations made by Carlson indicated similarly a concentration of approximately 71 per cent.

Since the experiments with rabbits involved very small doses, it was found advisable to reduce the concentration of the ethereal solution in order to facilitate measurement of doses. A 6.7 per cent solution, therefore, was used in the later acute experiments with this species.

A number of experiments were performed in which the skins of rabbits were exposed to various single doses of uranyl nitrate hexahydrate, followed 4 hr later by washing of the exposed dermal area with various detergents. With the small numbers of animals used, the mortalities resulting from these exposures (see Sec. 3) were considered sufficiently similar to the mortalities resulting from similar exposures of the rabbits that were not subsequently washed to warrant their inclusion in the calculation of the general dosage-mortality curve for rabbits exposed to uranyl nitrate.

One group of guinea pigs was exposed to a 71 per cent solution of uranyl nitrate hexahydrate in water. Since the mortality within this group was identical with that within the group of guinea pigs exposed to equivalent doses of the compound in ethereal solution, they were included in the calculation of the general dosage-mortality curve for guinea pigs exposed to uranyl nitrate.

The following technique was employed in effecting dermal exposure in all cases: One or more days prior to exposure the fur was removed from the backs of the animals by means of clippers. It was found advisable to clip the animals at least 24 hr before exposure to allow time for subsidence of the temporary hyperemia caused by the clipping. Depilatories had been tried but were found to be injurious to the skins of the animals.

The solutions were measured accurately in graduated syringes. The total dose of the compound, in grams per kilogram, was calculated for each animal and translated into milliliters of the solution. The solution was then drawn into a syringe equipped with a blunted needle of large bore, the requisite number of milliliters was measured, and the solution was spread as evenly and as thinly as possible over a dermal area, which varied in size according to the size of the dose, care being taken to avoid extraneous scratches and abrasions. The vehicle was allowed to evaporate before the animal was returned to his cage.

A large number of control animals of each species was studied with respect to all the observations that were made on the animals that were acutely exposed (single doses). These animals were treated in exactly the same way as were the exposed animals, with the single exception that uranium was not present in the exposure vehicle. The fur was removed from their backs by means of clippers, and their bare skins were exposed to quantities of pure ether, which were roughly equivalent to the quantities of the uranium-containing ethereal solutions applied to the experimental animals.

It is obviously difficult to measure effectual dermal exposure quantitatively, and there are many possible sources of error in this method of administration.

For example, dried crystals of the uranyl nitrate were visible on the surface of the skin throughout the periods of observation. Moreover, this compound caused a visible coagulation necrosis of the superficial layers of the skin, resulting in a barrier that might inhibit prolonged absorption. Hence in no case was the total applied dose absorbed, and it was impossible to determine the fraction of the dose that was absorbed in individual cases. All data concerning dosage therefore apply to the dose applied to the skin and not to the dose absorbed through the skin.

Uniformity in the thickness of the layer of the compound in contact with the skin was impossible. Especially in the case of mice, for which a very large dose in proportion to dermal area was required, the solution, even when it covered a large portion of the body surface, was concentrated over a comparatively very small skin area. Moreover above a certain dose higher doses did not cover a proportionally larger area of the mouse.

Rats and mice after having been returned to their cages were able to remove, by rubbing and licking, a portion of the dried salt that remained on their backs. Since washing the exposed areas of the skins of rabbits did not alter the toxicity of a given dose, this voluntary washing action on the part of rats and mice probably had no significant effect on the effectual dose, as regards absorption through the skin.

This licking action, however, resulted in the ingestion of indeterminable quantities of the compound. Any additive effects from such potential oral intoxication could not be determined. No holder was found that for long periods would restrain the animals from washing themselves.

The date of exposure and the date of death, and therefore the time of survival of each individual animal, was recorded. A constant gain in weight was the best criterion for assuming that an animal was going to survive. Most of the animals were kept under observation until body weight had returned to a value at least as high as the body weight at the time of exposure. This involved a period of 30 days or less. Any animal that was known to have died accidentally or from any extraneous cause was excluded from the final data.

Because of the afore-mentioned limitations on precision, inherent in the measurement of toxicity from percutaneous absorption, a large degree of dispersion was observed with the use of small numbers of animals per dose group. For this reason it was necessary to perform several experiments for rabbits and guinea pigs before sufficient data could be obtained for statistically significant evaluation of the relations between dose and mortality. These quantitative studies were done for the acute exposures (single doses) to uranyl nitrate hexahydrate only.

The experimental results have been presented in summary form in Table 8.1.

The dosage-mortality curves (frequently called "characteristic" or "sigmoid" curves) for rabbits, guinea pigs, rats, and mice that were dermally exposed to uranyl nitrate hexahydrate in ethereal solutions, have been plotted in Fig. 8.1, where it may be seen at once that there was a distinct difference in susceptibility among species.

In order to fit the curves to the observed points, each dose was converted to the logarithm dose, and each percentage mortality was converted to probit mortality. Assuming a linear correlation between logarithm dose and probit mortality, a straight line (regression line) was fitted by the least squares method according to the procedure of Bliss.[3] The regression lines for the four species, together with their fiducial limits, within which 19 of 20 experimentally repeated determinations of the regression lines might be expected to fall, have been presented in Fig. 8.2.

Chi-squared analysis of the data indicated that the observed scatter of points above and below the fitted regression lines could have occurred by chance.

Table 8.1—Toxic Manifestations Following the Application of Uranyl Nitrate Hexahydrate in Ethereal Solution to the Skins of Experimental Animals

Dose, compound, g/kg	Dose, uranium, g/kg	No. of animals	Mortality, %	Max. daily av. body weight as % of normal	Max. daily av. urinary protein, mg %	Max. daily av. blood NPN, mg %	Average renal damage at autopsy	No. autopsied
Rabbits								
Control	0.0000	9	0	100	10	40		
0.0067	0.0032	8	0	93	140	40		
0.013	0.0064	28	7	92	180	90		
0.027	0.013	8	0	100	140	60		
0.034	0.016	20	20					
0.10	0.048	20	30					
0.13	0.064	18	56	89	580	220	2½+	4
0.16	0.076	5	40		290	180	1½+	3
0.17	0.080	20	40					
0.22	0.10	10	70	79	1,050	260		
0.24	0.11	5	80	82	340	330		
0.27	0.13	7	100					
0.31	0.15	10	100	78	1,050	290		
0.38	0.18	5	80	70	180	340	2½+	5

Table 8.1 — (Continued)

Dose, compound, g/kg	Dose, uranium, g/kg	No. of animals	Mortality, %	Max. daily av. body weight as % of normal	Max. daily av. urinary protein, mg %	Max. daily av. blood NPN, mg %	Average renal damage at autopsy	No. autopsied
				Guinea pigs				
Control	0.00	51	0	100	0			
0.67	0.32	76	8	98				
1.3	0.64	56	7	96			0	1
2.0	0.95	43	9	97				
2.7	1.3	15	41	82	370		0	1
4.0	1.9	38	40	86				
4.6	2.2	24	50	85			2+	2
5.4	2.5	16	63	93			3 ½+	2
7.4	3.5	16	75	76				
8.0	3.8	16	63	76				
10.4	4.9	35	91	82	530		2 ½+	2
18.8	8.9	19	95	68	600			
				Rats				
Control	0.00	15	0	100	0			
0.38	0.18	15	40	88			3+	4
0.56	0.27	15	47	86	100		3+	3
0.83	0.39	17	41	76	240		3+	3
1.3	0.60	17	35	83	180		1 ½+	3
1.9	0.89	17	59	76	260		4+	6
2.8	1.3	19	63	74	510		3 ½+	7
4.3	2.0	17	88	72	250		3 ½+	3
6.3	3.0	2	100				4+	1

The dose (LD_{50}) that might be expected to kill 50 per cent of the animals of a given species is used to characterize that species with respect to a given toxicological exposure. These LD_{50}'s for the four species dermally exposed to uranyl nitrate hexahydrate in ethereal solutions have been listed in Table 8.2. The dose in terms of uranium, as well as in terms of $UO_2(NO_3)_2.6H_2O$, which contains 47.4 per cent of uranium, has been listed.

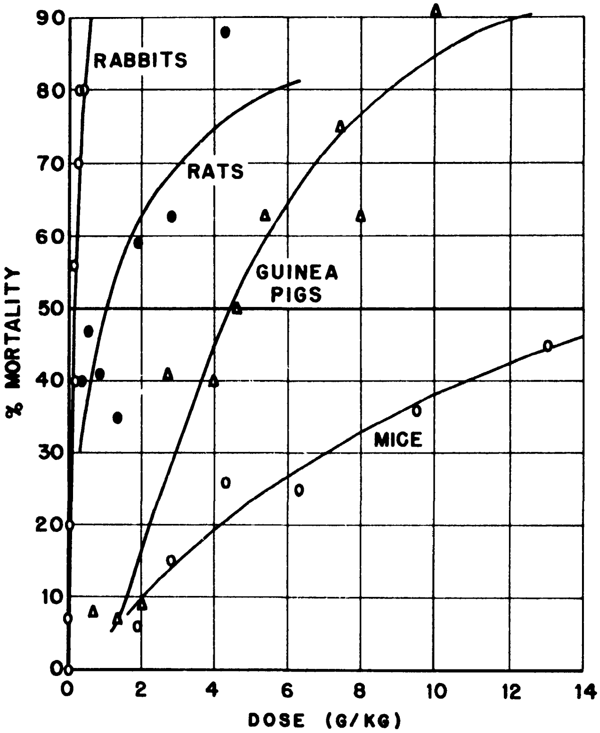

Fig. 8.1—Dosage-mortality curves for various species dermally exposed to uranyl nitrate hexahydrate in ethereal solutions.

It is of interest to note, from inspection of Table 8.2 or of Fig. 8.2, that in no case do the fiducial limits of the LD_{50}'s of any two species overlap. There is therefore a significant species difference in susceptibility to this compound.

In the case of rats, unfortunately, an insufficient number of dose levels below the LD_{50} were employed. For this reason the atypical form of the dosage-mortality curve for rats cannot be explained. Such a distribution of dosages resulted in an average dose that was higher than the LD_{50} and therefore the range of greatest trust is above the range of the LD_{50}, i.e., the standard error of the LD_{50} is larger than it would be if the LD_{50} coincided with the average dose.

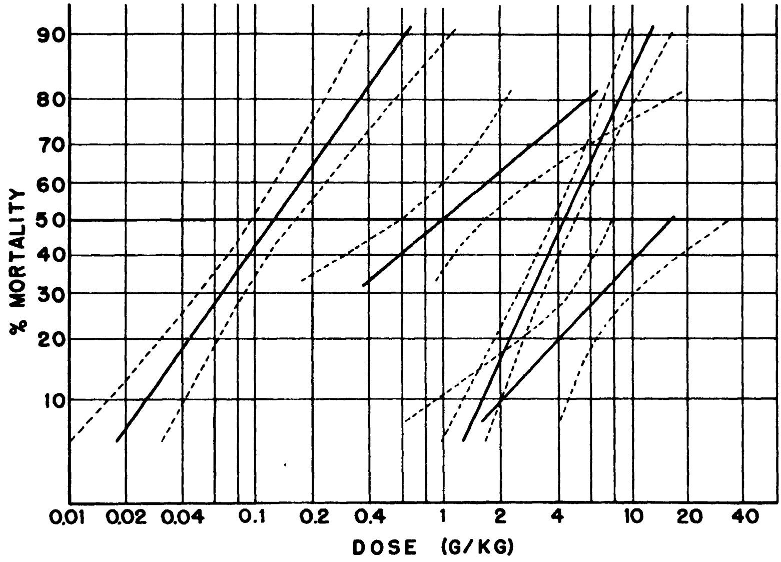

Fig. 8.2—The logarithm of the dose has been plotted against probit mortality to give the regression lines for (from left to right) rabbits, rats, guinea pigs, and mice, dermally exposed to uranyl nitrate hexahydrate in ethereal solution. The solid lines are the fitted regression lines, and the broken lines are their fiducial limits for odds of 19 in 20.

On the other hand all the dosages applied to the mice lay below the LD_{50}, resulting in a rather nebulous value for the LD_{50}, with a very wide margin of error. The highest dose applied to the skins of mice, 13 g/kg, covered a very large portion of the total body surface and resulted in only 45 per cent mortality. The dose that might be expected to kill 25 per cent of a group of mice (LD_{25}) represented a value close to the average dose applied in these experiments and therefore can be reported with greater accuracy than can the LD_{50}. Its calculated value was 5.4 g/kg, and, for odds of 19 in 20, it lay within the fiducial limits, 3.7 to 8.2 g/kg (an average variation of ±42 per cent).

In order to study the distribution of the time of survival following single exposures, the total number of deaths occurring in all dose groups of a species was recorded for each day following exposure and was then expressed as percentage of the total number of deaths occurring during the entire period of observation. This percentage, when plotted against the number of days following exposure, indicated the period during which mortality was highest and allowed a valid comparison of species.

Table 8.2 — Relative Susceptibility of Different Species to Uranyl Nitrate Hexahydrate Applied to the Skin in Ethereal Solutions

Species	Dose, g/kg				Av. variation of LD_{50}, %	Ratio of LD_{50}'s
	$UO_2(NO_3)_2.6H_2O$		Uranium			
	LD_{50}	Fiducial limits	LD_{50}	Fiducial limits		
Rabbits	0.125	0.095 - 0.165	0.059	0.045 - 0.078	±28	1
Rats	1.04	0.63 - 1.73	0.49	0.30 - 0.82	±53	8
Guinea pigs	4.44	3.88 - 5.09	2.11	1.84 - 2.41	±14	36
Mice	16	6.7 - 33.9	7.6	3.2 - 16.1	±85	128

The distribution of the time of survival following percutaneous poisoning with uranyl nitrate hexahydrate has been illustrated graphically in Fig. 8.3, where it may be seen that the modal time of survival for all species was between 5 and 7 days following exposure. Also in every case the first fatality occurred 2 or 3 days following exposure, and in general the form of the distribution was quite similar for all species. A notable exception was the occurrence of "stragglers" among the guinea pigs, some of which survived as long as 28 days before succumbing. The time of survival tended roughly to increase with decrease of the dosage.

In order to determine whether the mortality within any particular weight range of species differed significantly from the mortality of the species in general, the guinea pigs, rats, and mice that had been dermally exposed to uranyl nitrate hexahydrate were divided into groups according to body weight, and the mortalities for each weight group were calculated. On subjecting the data to the χ^2 test (statistics by Tiedeman), the lowest p value of the three experiments was found to be 0.38, for mice. This indicated that a χ^2 as large as or larger than the one observed would be expected to occur by chance in 38 out of 100 repetitions of this experiment if mortality were independent of weight. In view of these large p values, it is evident that there was no significant difference in susceptibility for any particular range of body weight. The rabbits were not tested in this respect because they were mostly adult animals of approximately the same body weight.

(b) Uranium Tetrachloride. In experiments designed to compare the toxicity of uranium tetrachloride, UCl_4, with that of the nitrate hexahydrate, three groups of guinea pigs were exposed to uranium tetrachloride in a 40 per cent aqueous solution.

The fur was removed from the backs of the animals, and their skins were exposed according to the technique described for uranyl nitrate hexahydrate (Sec. 1.1 preceding).

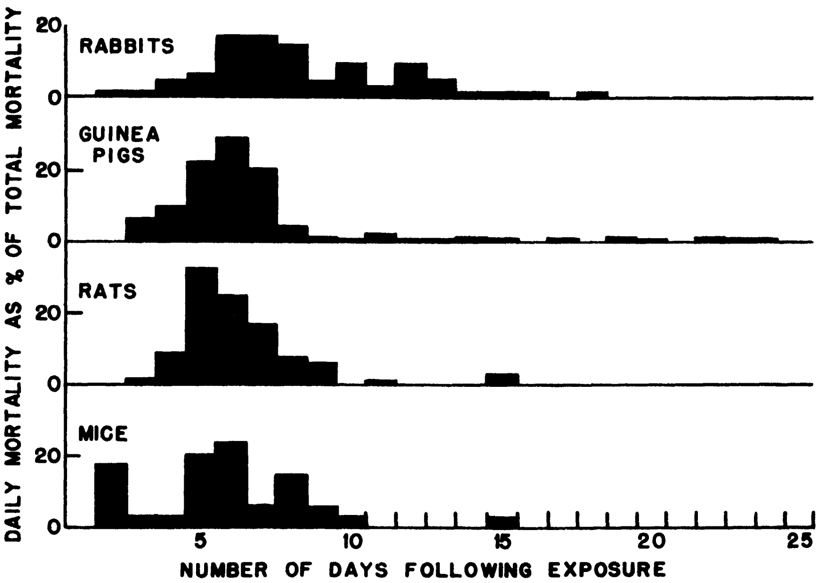

Fig. 8.3 — Frequency distribution of the time of survival following dermal exposure to uranyl nitrate hexahydrate in ethereal solutions. Comparison of species.

The mortalities observed from these exposures of guinea pigs to uranium tetrachloride (see Table 8.3) were compared with the expected mortalities from similar doses of uranyl nitrate hexahydrate in order to give some indication of the relative toxicities of the two compounds. The mortalities to be expected from the dermal exposure of guinea pigs to uranyl nitrate hexahydrate were calculated from the formula for the regression line illustrated in Fig. 8.2. The X^2 test, as suggested by Bliss,[4] indicated, when applied to these two sets of data (Table 8.4), that the mortalities from exposure to uranium tetrachloride were probably not homogeneous with the mortalities from exposure to the nitrate hexahydrate. If the two compounds are compared in the same way on the basis of uranium content, it may be seen that the dermally applied uranium tetrachloride was significantly less toxic for the guinea pigs than was the uranyl nitrate hexahydrate.

The similarity between the distributions of the time of survival following the percutaneous poisoning of guinea pigs with uranyl nitrate hexahydrate and with uranium tetrachloride has been illustrated in Fig. 8.4.

Table 8.3—Toxic Manifestations Following the Application of Uranium Tetrachloride in Aqueous Solution to the Skins of Guinea Pigs

Dose, g/kg		No. of animals	Mortality, %	Min. daily av. body weight, % of normal	Max. daily av. urinary protein, mg %
Compound	Uranium				
Control	0.0	51	0	100	0
1.8	1.1	21	24	90	120
3.4	2.1	21	19	90	90
6.7	4.2	21	48	80	130

Table 8.4—Relative Mortalities from Equivalent Doses of $UO_2(NO_3)_2.6H_2O$ and UCl_4*

Amount of UCl_4 given, g	Observed mortality, %	Expected mortality from an equal weight of $UO_2(NO_3)_2.6H_2O$, %	Expected mortality from an equivalent weight of $UO_2(NO_3)_2.6H_2O$, %
1.75	24	12	21
3.40	19	37	51
6.70	48	70	80
		p values* (vs. col. 2)	
		0.04	<0.01

*p values were obtained by Tiedeman and assistants employing the chi-square method.

(c) Other Compounds. The remaining compounds that were applied to the skins of rabbits were investigated in a qualitative way only, and it is possible to classify them only roughly according to relative toxicity from the observed mortalities and other toxic manifestations listed in Table 8.5.

Lanolin (adeps lanae hydrosus, U.S.P.) was used as a vehicle for those compounds that were insoluble or relatively insoluble in water or ether [UO_2, U_3O_8, UO_3, UO_4, UF_4, $Na_2U_2O_7$, and $(NH_4)_2U_2O_7$], and for the sake of comparison the water-soluble UO_2F_2 and UCl_4 were also applied in suspension in lanolin. A quantity of the compound sufficient to allow 1 to 2 g/rabbit was mixed with approximately half its weight of lanolin. This resulted in an approximately 65 per cent suspension.

These suspensions were divided by means of a spatula, as evenly as possible, into equivalent doses. Each dose was then applied by hand to the back of a rabbit and was spread over the skin with the gloved fingers.

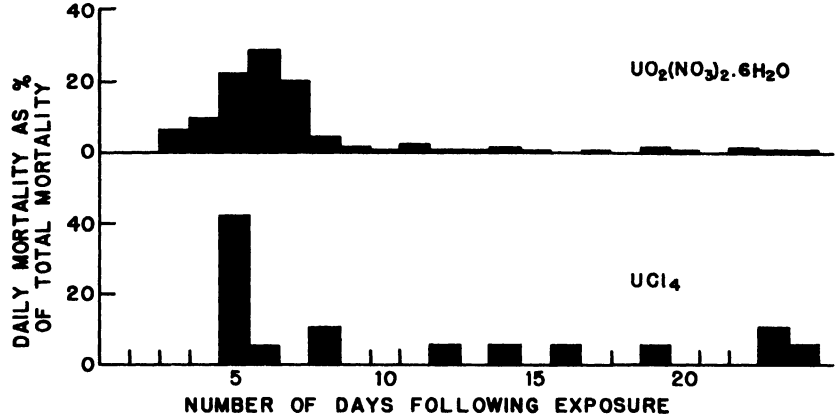

Fig. 8.4 — Frequency distribution of the time of survival of guinea pigs following dermal exposure. Comparison of uranyl nitrate hexahydrate and uranium tetrachloride.

Uranium tetrachloride, UCl_4, and uranium pentachloride, UCl_5, were applied as dry powders directly to the backs of two groups of rabbits, respectively, and were spread over the skin with the gloved fingers. Because of their highly hygroscopic properties, however, these two compounds rapidly dissolved in the moisture absorbed from the air and became in effect concentrated aqueous solutions.

Uranyl fluoride, UO_2F_2, was applied in a 50 per cent aqueous solution to the skin of one group of rabbits.

The date of exposure of each group of animals was recorded.

The date of death and therefore the time of survival of each individual animal was recorded. A constant gain in weight was the best criterion for assuming that an animal was going to survive. Most of the animals were kept under observation until body weight had returned to a value at least as high as the body weight at the time of exposure. This involved a period of 30 days or less. Any animal that was known to have died accidentally or from any extraneous cause was excluded from the final data.

Table 8.5—Toxic Manifestations Following the Application of Various Uranium Compounds to the Skins of Rabbits

Compound	Concentration, %	Vehicle	Dose per rabbit		No. of animals	Mortality, %	No. of days following exposure			Local effect*	Urinary protein, mg%	
			Compounds, g	Uranium, g			First death	Last death	Observed until		Max. daily av.	Range of individual maxima
$UO_2(NO_3)_2.6H_2O$	71	Ether	1.4	0.7	6	100	4	6	6	3	†	
UO_2F_2	65	Lanolin	1.0	0.8	6	100	5	8	8	0	1,500	120-2,000
UO_2F_2	50	Water	1.0	0.8	6	83	5	10	30	0	210	70-600
UCl_4	65	Lanolin	2.0	1.3	6	100	4	9	9	1	500	100-1,000
UCl_4	100	None	0.5	0.3	4	50	10	14	23	0	150	80-350
UCl_5	100	None	1.0	0.6	6	67	7	15	21	2	575	100-1,200
UO_3	65	Lanolin	1.0	0.8	6	67	6	7	30	0	350	250-550
$Na_2U_2O_7$	65	Lanolin	1.0	0.8	6	67	5	16	31	0	230	60-550
$(NH_4)_2U_2O_7$	65	Lanolin	1.0	0.8	6	33	3	11	30	0	160	20-350
UO_4	65	Lanolin	1.0	0.8	6	0			33	0	80	60-150
UF_4	65	Lanolin	1.0	0.8	6	0			34	0	60	40-120
UO_2	65	Lanolin	1.0	0.8	6	0			28	0	50	40-140
U_3O_8	65	Lanolin	1.0	0.8	6	0			28	0	50	40-140

*The degree of effect is indicated by numbers which are defined as follows: 3, immediate coagulation necrosis and erythema of moderate degree; 2, coagulation necrosis and erythema after 7 to 9 days; 1, erythema of short duration only; 0, no damage at any time.

†See Table 8.1.

From the results obtained with only six animals per compound it appears that, as might be expected, the extremely water-soluble compounds (uranyl nitrate, uranyl fluoride, uranium tetrachloride, and uranium pentachloride) were the most toxic; the slightly soluble compounds (uranium trioxide, sodium diuranate, and ammonium diuranate) were of intermediate toxicity; and the insoluble compounds (uranium tetrafluoride, uranium dioxide, uranium peroxide, and uranium trita-octoxide) were nontoxic when applied to the skins of rabbits.

1.2 Body Weight. Most of the animals that survived the percutaneous poisoning with uranium compounds suffered, nevertheless, a temporary loss of weight, which was proportional, in general, to the size of the dose.

The body weights were recorded, at regular intervals following exposure, for all the species acutely and chronically exposed to uranyl nitrate hexahydrate and for the guinea pigs exposed to uranium tetrachloride (Sec. 1.1). Each animal was weighed at least once prior to exposure and at intervals thereafter at least until a return toward normal was evidenced.

Each time an animal was weighed its body weight was translated into percentage of its preexposure weight.

Assuming the average of the body weights (expressed as percentage of preexposure weight) of the control animals on any particular day following exposure to be "normal" for that day, the average body weight for each dose group was expressed as percentage of normal, for each day, according to the following formula:

$$\frac{\mathrm{Nc}\sum\left(\frac{\mathrm{We}}{\mathrm{We_0}}\right)}{\mathrm{Ne}\sum\left(\frac{\mathrm{Wc}}{\mathrm{Wc_0}}\right)} \times 100$$

where We = observed weight of the exposed animal, for a given day.
$\mathrm{We_0}$ = initial weight of the exposed animal.
Wc = observed weight of the control animal, for the same day.
$\mathrm{Wc_0}$ = initial weight of the control animal.
Ne = number of exposed animals.
Nc = number of control animals.

(a) Uranyl Nitrate Hexahydrate. Correlation between the dose of uranyl nitrate hexahydrate and the magnitude of weight loss was illustrated by plotting the dose against the minimal mean body weight (as percentage of "normal") of the dose group (Table 8.1 and Fig. 8.5).

The various dose groups within each species were grouped together into three or four larger groups designated, respectively, as high, medium, and low dose groups. The mean body weights of these larger groups, expressed as above, were then plotted against the number of days following exposure. This gave graphic indication of the periods during which body weight was lowest. Data were sufficient to express this variation of body weight with time for all the species acutely exposed to the nitrate hexahydrate and for the guinea pigs exposed to the tetrachloride.

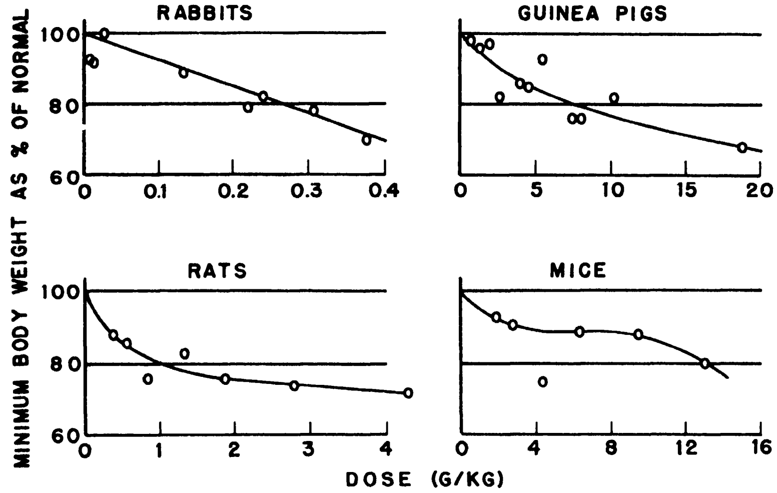

Fig. 8.5—Correlation between the size of the dose and the maximal mean decrease in body weight observed in various species following dermal exposure to uranyl nitrate hexahydrate in ethereal solutions. "Normal" is here defined as the mean weight of the control animals of a species on any given day.

The body weight reached a minimal value in from 6 to 10 days following exposure and then began to rise again toward preexposure levels. In Fig. 8.6 the mean growth curve for the control animals of each species has been linearized on the 100 per cent abscissa. The mean curves for the various groups of animals exposed to uranyl nitrate hexahydrate are therefore represented as percentages of this normal growth curve. The character of the weight curves is somewhat different for each species.

In the case of guinea pigs, with the exception of the smallest dose groups, the mean body weights reached a minimum and then began to rise at a rate nearly equal to that of the controls, and, therefore, although they returned to their individual preexposure levels, they never reached normal. Hence the postminimum portions of the curves in Fig. 8.6 are represented as horizontal lines roughly parallel with the abscissa. The body weights of the lowest dose groups of guinea pigs, however, fell not very far below those of the controls and proceeded to catch up with them again within 14 days.

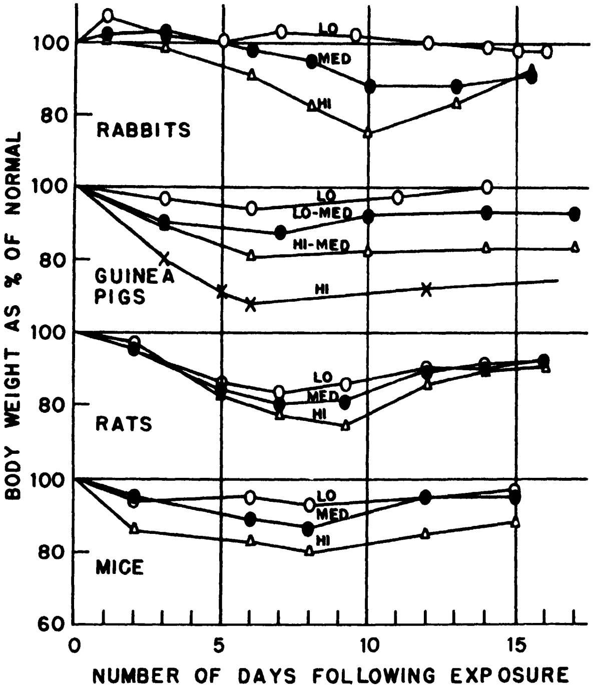

Fig. 8.6 —The daily variation in body weight of various species following dermal exposure to uranyl nitrate hexahydrate in ethereal solutions. "Normal" is here defined as the mean weight of the control animals of a species on any given day. The 100 per cent abscissas therefore represent the linearized growth curves of the control animals.

The mean weight curves of the various groups of rats exposed to uranyl nitrate hexahydrate returned almost, but not quite, to the level of the normal growth curve of the control rats and then continued to rise at a rate parallel to it.

Within 21 days following exposure, the mean weight curves of the exposed rabbits and mice reached, and in some cases even surpassed, the normal growth curves of their respective controls. The original nonlinearized growth curve of the control rabbits, however, was itself essentially a horizontal line because the rabbits were for the most part fully grown animals.

(b) Uranium Tetrachloride. There was little difference between the character of the weight curves of guinea pigs exposed to uranyl nitrate hexahydrate and to uranium tetrachloride. Their similarity is illustrated in Fig. 8.7. Minimal values were listed in Table 8.3.

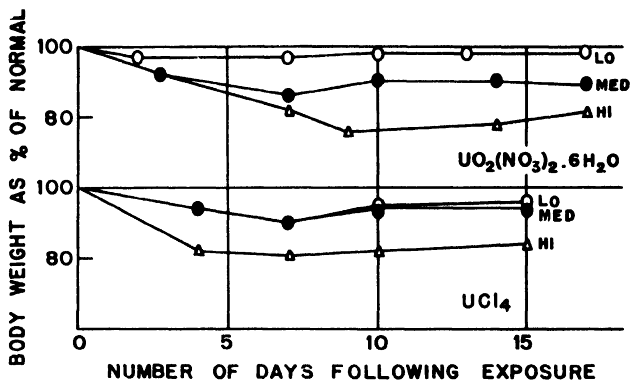

Fig. 8.7 — The daily variation in body weight of guinea pigs following dermal exposure. Comparison of uranyl nitrate hexahydrate and uranium tetrachloride. "Normal" is here defined as the mean weight of the control animals of a species on any given day. The 100 per cent abscissas therefore represent the linearized growth curves of the control animals.

1.3 Urinary Changes. Urinary protein, in milligrams per cent, was recorded at various intervals following exposure for all the species exposed to all the compounds. The rabbits and guinea pigs were kept in metabolism cages, and 24-hr samples of urine were collected. Separate samples were collected for each rabbit, and, since five to eight guinea pigs were housed in one cage, a pooled sample of their urine was tested. The mice and rats were placed in small metabolism cages for short intervals, and 2- or 3-hr samples were collected. Samples were obtained from individual rats, and pooled samples from every six or seven mice.

Quantitative tests for urinary protein were made according to the method of Kingsbury, Clark, *et al.*[5]

(a) Uranyl Nitrate Hexahydrate. The individual values within each dose group were averaged for each day, and the maximum obtained during the experiment was recorded in Table 8.1.

There was a rough positive correlation between the size of the dose of uranyl nitrate hexahydrate and the maximal degree of proteinuria in all species, but the scatter of points was sufficiently wide so as not to warrant a graphic representation of the correlation. The proteinuria observed in those groups of rabbits receiving the lowest doses (0.0067, 0.013, and 0.027 g/kg) was considerably lower than in the other groups, so a separate time-series curve has been plotted for these three low dose groups in Fig. 8.8. For each of the remaining three species, however, the observed values for urinary protein in all the dose groups have been averaged and plotted in Fig. 8.8 as the mean time-series curve for the species.

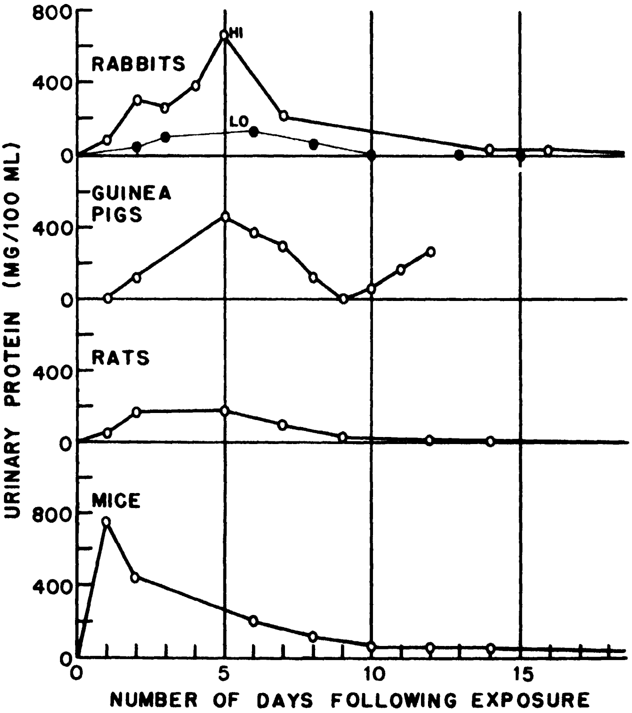

Fig. 8.8—The daily variation in the mean urinary protein of various species following dermal exposure to uranyl nitrate hexahydrate in ethereal solutions.

In all species, urinary protein became significantly positive by the first or second day following exposure. In the case of rabbits, guinea pigs, and rats, the mean urinary protein was highest on the fifth day following exposure, just at the beginning of the period when mortality was highest. The proteinuria of the rabbits and the rats returned to within a normal range by the fourteenth day following exposure, whereas that of the guinea pigs showed a peculiar secondary rise after having returned to normal on the ninth day. This secondary rise in urinary protein, which was unfortunately not followed beyond the twelfth day, may have been related in some way to the delayed deaths seen among guinea pigs (Fig. 8.3). The mean curve for the urinary protein of mice was atypical, reached a maximum on the first day following exposure, and then fell rapidly to a very low value by the tenth day but remained thus slightly positive for 40 to 50 days following exposure.

The daily urinary glucose, the daily urinary volume, and the urinary pH were recorded in some of the experiments with rabbits exposed to the nitrate hexahydrate. None of these, however, showed any correlation with the size of the dose or with the length of time following exposure. These data have, therefore, been omitted from this report.

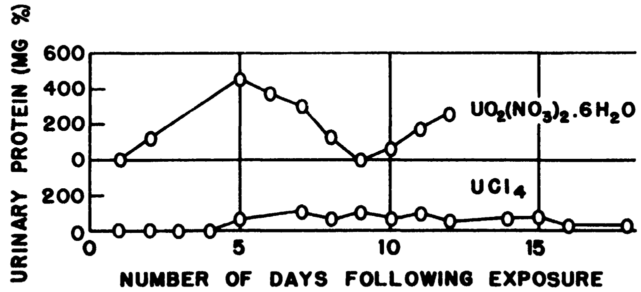

Fig. 8.9—The daily variation in the mean urinary protein of guinea pigs following dermal exposure. Comparison of uranyl nitrate hexahydrate and uranium tetrachloride.

(b) Uranium Tetrachloride. As with the nitrate, there was little correlation between the dose of uranium tetrachloride applied to the skin of guinea pigs and the observed degree of proteinuria (Table 8.3). The time-series curve (Fig. 8.9), however, was distinctly different from that resulting from exposure to the nitrate hexahydrate. The mean urinary protein of the guinea pigs exposed to uranium tetrachloride did not become positive until the fifth day following exposure, reached a maximum only one-fourth as high as that of the guinea pigs exposed to the nitrate hexahydrate, remained thus moderately elevated until the fifteenth day following exposure, and was still slightly positive on the twenty-fourth day, when observations were discontinued.

(c) Other Compounds. The dermal exposure of rabbits to the other compounds of uranium resulted in mean time-series curves (Figs.

8.10 and 8.11) for urinary protein that were in general qualitatively similar to that for uranyl nitrate hexahydrate. The height of the maxima, however, varied presumably according to the relative toxicity of the compound (Table 8.5). Uranium tetrafluoride, uranium peroxide, uranium dioxide, and uranium tritaoctoxide produced no elevation of urinary protein above the normal range for rabbits.

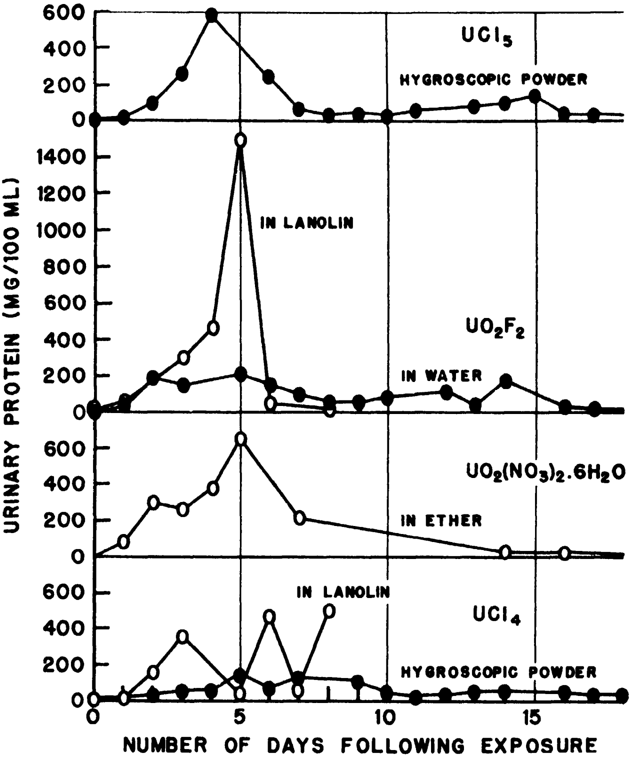

Fig. 8.10—The daily variation in the mean urinary protein of rabbits following dermal exposure. Comparison of various highly water-soluble compounds and of the type of vehicle.

1.4 Nonprotein Nitrogen of the Blood. It was practical to collect samples of blood from only the rabbits. Nonprotein nitrogen in milligrams per cent of blood was determined at various intervals following exposure for the rabbits exposed to uranyl nitrate hexahydrate. Analyses were made by Bumaster and assistants, according to the Folin and Wu principle as modified by Hawk and Bergeim.[6]

The individual values within each dose group were averaged for each day, and the maximum obtained during the experiment (see Table 8.1) was plotted against the dose to illustrate the correlation between the dose and the degree of azotemia (Fig. 8.12). The same maximal NPN values were plotted against mortality (Fig. 8.13).

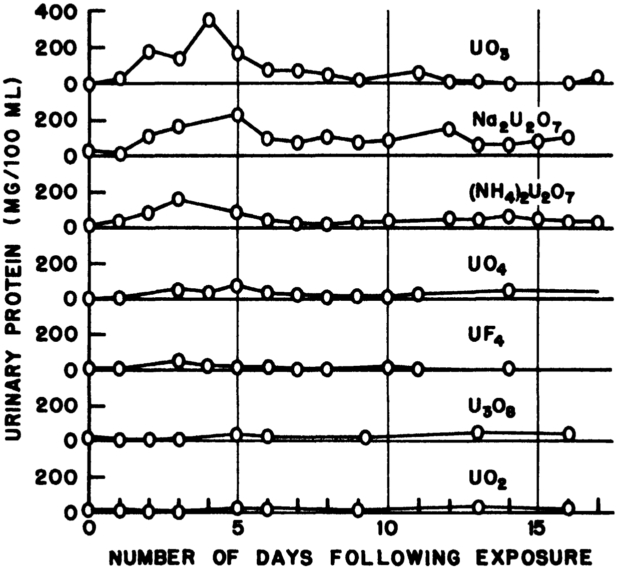

Fig. 8.11—The daily variation of the mean urinary protein of rabbits following dermal exposure to compounds of uranium in suspension in lanolin. Comparison of various insoluble and relatively insoluble compounds.

The correlation between the size of the dose and the mean degree of azotemia was good, but the correlation between azotemia and mortality was even better, being an almost perfect linear function between 0 and 70 per cent mortality. Thus the mortality of a given group of rabbits can be predicted with a fair degree of accuracy from the maximal value observed for the mean blood NPN of the group, by reference to Fig. 8.13.

The blood NPN in those groups of rabbits receiving the lowest doses (0.0067, 0.013, and 0.027 g/kg) did not rise significantly above normal. Separate mean time-series curves therefore have been plotted for these and the remaining dose groups in Fig. 8.14.

The mean NPN curve trailed the mean urinary-protein curve by a period of several days, reaching a significant value on about the third day after exposure, attaining a maximum (approximately 250 mg %) on the ninth day, and then falling rapidly to within the normal range by the fifteenth day. It is of interest to note that the NPN curve (Fig. 8.14) was still rising while the urinary-protein curve (Fig. 8.8) had passed its maximum and was rapidly falling. The period (fifth to tenth days following exposure) when NPN was highest was also the period when mortality was highest.

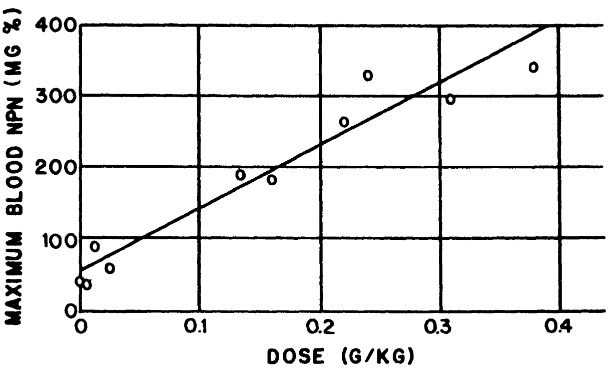

Fig. 8.12—Correlation between the size of the dose and the maximal mean NPN of the blood of rabbits following dermal exposure to uranyl nitrate hexahydrate in ethereal solutions.

1.5 Uranium Content of the Blood. Rats were sacrificed, in groups of four, at various intervals after dermal exposures to uranyl nitrate in ethereal solution, and pooled samples of blood were collected, avoiding all possible contamination. Significant amounts of uranium (0.2 to 1.0 μg/ml) were found, by the fluorophotometric method of Neuman[7] (see also Chap. 2), to be present within 5 min after exposure and as long as 12 hr following exposure, although there was no correlation between the uranium content of the blood and the length of time after dermal exposure, in contrast to that seen after intravenous injection (see Chap. 11).

1.6 Pathology. The kidneys and lungs of most of the rabbits that died were examined grossly for evidences of uranium poisoning. Tissues from a number of the rabbits, rats, mice, and guinea pigs that were exposed to uranyl nitrate hexahydrate were examined microscopically by Metcalf. Animals that were sacrificed at the end of an experiment, as well as those that died during the experiment, were examined. Evidences of typical uranium poisoning and of intercurrent pathology, which might have been an extraneous cause of death, were recorded.

All the animals that died following the dermal exposure to uranium compounds and that were examined microscopically showed the typical renal damage of uranium poisoning (see Chap. 4). Some of the survivors that had shown clinical and chemical evidence of uranium poisoning, but had recovered, were sacrificed and examined microscopically after 20 to 30 days. The kidneys of these animals were essentially normal, evidencing a remarkable ability for repair.

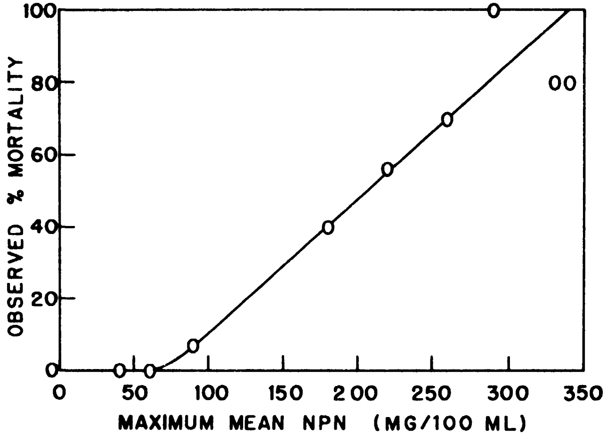

Fig. 8.13—Correlation between the maximal mean NPN of the blood of rabbits, following dermal exposure to uranyl nitrate hexahydrate in ethereal solutions, and the observed mortality.

1.7 Local Irritation. Local irritation at the site of application of the various compounds to the skins of rabbits was evaluated roughly according to the degree of erythema, of coagulation necrosis, or of ulceration.

Only three of the compounds investigated caused any visible change in the skin itself. Uranyl nitrate hexahydrate, in ethereal or aqueous solution, produced a superficial coagulation necrosis within a few hours, followed rapidly by a moderate hyperemia of the areas peripheral to and underlying the eschar. Repair was grossly complete, in all cases, within a week. Sections of such skins were examined by Metcalf and revealed, microscopically, a mild degree of chronic inflammation.

A similar but delayed effect was seen 7 to 9 days following the application of the powdered hygroscopic uranium pentachloride to the skins of the rabbits.

Uranium tetrachloride in suspension in lanolin caused a moderate, local erythema of the skin at the site of application, which disappeared in 1 or 2 days. The powdered hygroscopic uranium tetrachloride, when applied directly to the skin, produced no visible change.

None of the other compounds, in any form, produced any visible local change in the skin.

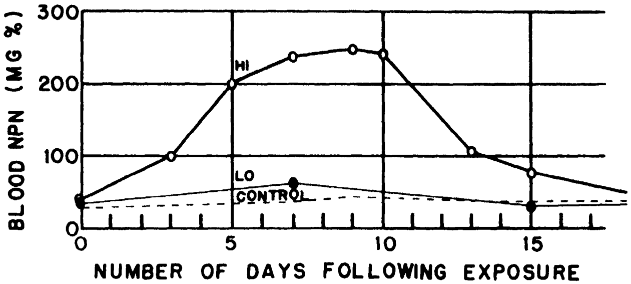

Fig. 8.14—The daily variation in the mean blood NPN of rabbits following dermal exposure to uranyl nitrate hexahydrate in ethereal solutions.

1.8 Clinical Changes. A number of the rabbits were followed, incidentally, with respect to clinical changes during the course of poisoning.

The first signs of poisoning in rabbits, which occurred the second or third day after exposure, were decreased food and water intake, accompanied by decrease in body weight. Soon the animals became extremely irritable and developed hyperactive reflexes, and 2 to 3 days preceding death they became weak and emaciated, with a disturbance of equilibrium and loss of reaction to stimuli. Eventually the hind legs became rigidly extended, and the animals no longer had the ability to use these limbs. Just before death the front legs became involved similarly but to a milder degree. During the last 2 days the body temperatures fell from the normal of 38 to 39°C to values between 34 and 36°C. Respiration eventually ceased, sometimes immediately preceded by convulsions.

The clinical picture of poisoning in guinea pigs was that of anorexia followed by progressive and extreme cachexia.

2. CHRONIC POISONING FROM URANYL NITRATE HEXAHYDRATE

2.1 Mortality. Quantitative studies of the relationships between dosage and mortality were not undertaken for the repeatedly exposed animals. Instead, more attention was directed to those doses that might be expected to produce very low mortalities.

The chronically exposed rabbits were exposed to a 71 per cent ethereal solution and the guinea pigs to a 13 per cent ethereal solution.

The backs and bellies of the guinea pigs to be subjected to chronic exposures were alternately clipped and painted throughout the experiment. They were weighed before every other exposure and the dose, in grams per kilogram, was calculated each time according to the animal's most recent weight and converted into the number of milliliters of the ethereal solution. The two highest chronic-dose groups were exposed three times per week, and the remaining four groups were exposed six times per week.

Table 8.6—Comparison of Mortalities Resulting from Acute and Chronic Poisoning of Guinea Pigs by Dermal Exposure to $UO_2(NO_3)_2.6H_2O$ in Ethereal Solution

No. of animals	Chronic poisoning			Acute poisoning, expected mortality, % (4)
	Single dose, g/kg (1)	Cumulative dose, g/kg (2)	Observed mortality, % (3)	
21	1.3	17	38	96
21	0.80	10	14	86
50	0.34	7.0	8	72
60	0.10	2.3	12	21

Four of the dose groups were repeatedly exposed until body weights and other clinical evidences indicated that no further mortality was to be expected. Observations on the remaining two groups were discontinued as soon as 5 per cent mortality was exceeded.

Owing to the slower rate of growth of rabbit hair, it was necessary to clip only once every week the backs of the rabbits to be subjected to chronic exposures. They were exposed five times per week, some at the same site each time and others at a different site each time.

Since a very small number of animals was used, there were no quantitative data on the mortality of rabbits subjected to chronic exposure. Chronic exposures of rats and mice were not investigated.

In Table 8.6, the mortality of guinea pigs resulting from the cumulative dose from repeated exposures has been compared with the mortality to be expected from application of the same quantity of the compound in a single dose, as calculated from the formula for the regression line that was obtained for guinea pigs in Fig. 8.2. From the consistently lower mortalities in column (3) of Table 8.6, it can be seen that, within the range of the doses that have been studied, the guinea pigs tolerated a given dermal exposure to uranyl nitrate hexahydrate much better when given in repeated small fractions than when given in a single exposure.

The relationship between the dose and the time required to exceed 5 per cent mortality has been illustrated in Table 8.7. With one discrepancy, namely, 0.13 g/kg, a general decrease in the time required to exceed 5 per cent mortality was noted as the dose was increased. A similar trend may be seen in the rabbits by comparing the time of survival with the dose received (Table 8.8).

Table 8.7 —Number of Days Required to Exceed 5 Per Cent Mortality upon Repeated Exposure of Guinea Pigs to Various Doses of $UO_2(NO_3)_2.6H_2O$ in 71 Per Cent Ethereal Solution

No. of animals	Single dose, g/kg	No. of doses per week	No. of exposures at 5% mortality	Cumulative dose at 5% mortality	Day that 5% mortality is exceeded
21	1.3	3	4	5.4	7th
21	0.80	3	5	4.0	10th
50	0.34	6	11	4.0	12th
50	0.20	6	10	2.2	13th
50	0.13	6	6	0.80	7th
60	0.10	6	13	1.3	15th

Four of the groups of guinea pigs were kept under observation until body weights and other clinical evidences suggested that no further mortality was to be expected (24 to 27 days). Actually no deaths were observed later than 20 days following exposure. The period therefore during which mortality from repeated exposures occurred did not exceed the period of mortality from acute exposure (see Fig. 8.3).

2.2 Body Weight. During chronic exposure of the skin to uranyl nitrate hexahydrate a fall, or at least a retardation of gain, in body weight was observed. Since there were no control animals, these body weights, in contrast to those of the acutely exposed animals, were expressed merely as percentages of initial weights. The mean body weight, expressed thus, of each dose group of guinea pigs was plotted against the number of days following exposure. The body weights of the three rabbits that survived chronic exposure to uranyl nitrate hexahydrate (0.012, 0.012, and 0.024 g/kg, respectively) at multiple sites were averaged and similarly plotted.

Table 8.8—Time of Survival of Rabbits Repeatedly Exposed to Various Doses of $UO_2(NO_3)_2.6H_2O$ in 71 Per Cent Ethereal Solution (Five Doses per Week)

No. of animals	Single dose, g/kg	Av. no. of exposures at death	Av. cumulative dose at death	Av. time of survival, days
		Single site of exposure		
4	1.8	2	3.6	4 ½
4	0.82	2	1.6	5 ½
2	0.39	4	1.6	5 ½
2	0.19	4 ½	0.85	8 ½
2	0.12	4	0.46	8 ½
2	0.024	5	0.12	9
		24	0.58	*
2	0.012	24	0.29	*
		Multiple sites of exposure		
1	0.13	3	0.40	5
1	0.034	4	0.14	6
1	0.022	21	0.46	*
2	0.011	8	0.088	8

*Sacrificed at 32 to 35 days.

The two groups of guinea pigs receiving repeated doses of 0.13 and 0.20 g/kg, respectively, were sacrificed before any significant trend in body weight had become apparent. The mean-weight curves of the remaining four groups have been presented in Fig. 8.15. The groups receiving 0.10 and 0.34 g/kg, respectively, were exposed six times per week, and, though they were originally rapidly growing young animals, there was no change in the mean body weight following the first 8 or 10 exposures. Thereafter, despite the fact that exposures were continued, there was a gradual increase in mean body weight for as long as the observations were continued. The two groups receiving repeated doses of 0.8 and 1.3 g/kg, respectively, were exposed only three times per week but exhibited a distinct fall in body weight. The mean body weights of both groups, however, had returned to preexposure values by the twelfth day following the first exposure, despite the fact that exposures were continued.

The three surviving rabbits exposed to repeated small doses of uranyl nitrate hexahydrate exhibited a similar temporary fall in mean body weight, which returned to its preexposure level by the twenty-second day even though they had been exposed five times per week throughout the period (Fig. 8.17).

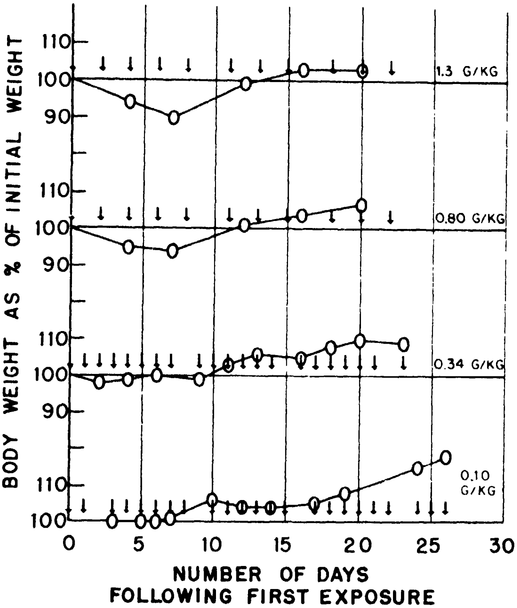

Fig. 8.15—The daily variation in mean body weight of guinea pigs following repeated dermal exposures to uranyl nitrate hexahydrate in ethereal solutions. Body weights are expressed in percentages of preexposure weights. Arrows indicate exposures.

2.3 Urinary Protein. Urinary protein was determined, as in Sec. 8.1, by the method of Kingsbury, Clark, *et al.*[5] Both guinea pigs and rabbits exhibited significant proteinuria during repeated dermal exposure to uranyl nitrate hexahydrate. That of the guinea pigs (Fig. 8.16) remained positive, though quantitatively irregular, for as long as observations were continued. The urinary protein of the rabbits (Fig. 8.17), however, returned to within a normal range on the twenty-fifth day (after 19 exposures) and remained thus for at least 10 days more, despite the fact that exposures were continued.

2.4 Local Irritation. The guinea pigs that were repeatedly exposed to small doses of uranyl nitrate hexahydrate exhibited the same superficial coagulation necrosis and inflammation of the epidermis as

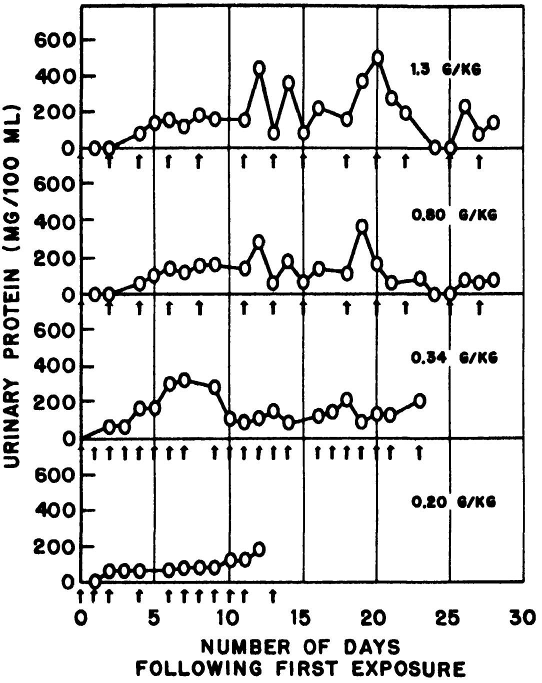

Fig. 8.16 — The daily variation in mean urinary protein of guinea pigs following repeated dermal exposures to uranyl nitrate hexahydrate in ethereal solutions. Arrows indicate exposures.

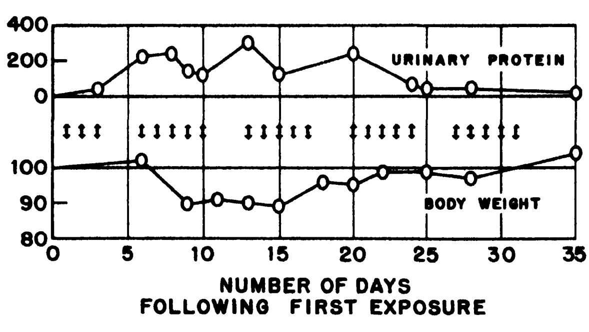

Fig. 8.17 — The daily variation in mean body weight and in mean urinary protein of rabbits following repeated dermal exposures to uranyl nitrate hexahydrate in ethereal solutions. The ordinate of the upper curve is urinary protein in milligrams per 100 ml, and the ordinate of the lower curve is body weight as percentage of preexposure weight. The arrows indicate exposures.

was seen in those exposed to single doses (Sec. 1). Their skins were in a constant state of encrustation and desquamation accompanied by rapid regeneration from beneath. The rabbits that were repeatedly exposed at multiple dermal sites exhibited an effect similar to that seen after single acute exposures, but those rabbits that were repeatedly exposed at the same site developed severe dermal ulcers after five to ten applications of the compound.

3. ATTEMPTS TO PROTECT AGAINST THE PERCUTANEOUS ABSORPTION OF URANYL NITRATE

A number of experiments were performed to study the effect of (1) detergents and (2) protective coatings on the course of poisoning following the dermal exposure of experimental animals to uranyl nitrate hexahydrate.

In the first type of experiment, rabbits and guinea pigs were dermally exposed to known amounts of the compound in ethereal solution, and 4 hr later the sites of exposure were washed thoroughly with one or another type of detergent. Groups of rabbits were washed with water, a 1 per cent aqueous solution of soap, and a 2 per cent aqueous solution of sodium bicarbonate, respectively. One group of guinea pigs was washed with water and another with a cosmetic preparation of cleansing cream.

In the second type of experiment, the skins of guinea pigs were coated with one of several common fatty preparations or commercial protective creams, and the skins of rabbits were painted with aqueous solutions of one of several chemical compounds, prior to exposure in each instance. The ethereal solution was then applied on top of the "protective" coating, and the skins of the animals were washed thoroughly with water 4 hr later.

The results have been summarized in Tables 8.9 and 8.10 where the observed and expected mortalities have been compared by the method of Bliss.[4] With the numbers of experimental animals employed, none of the substances tested by either method was observed to afford any statistically significant decrease in mortality below expected values. One substance, commercial cream No. 2, containing cocoa butter, significantly increased the mortality of guinea pigs above the expected value.

The apparently significant increases in mortality seen following the washing with soap and with bicarbonate, and following the precoating with picric acid and with tannic acid, cannot be held to be significant because of the very small numbers of animals employed, together with the observed 100 per cent mortalities, which cannot be made to fit properly into Bliss's method for the comparison of mortalities.[8]

Table 8.9—Effect of Subsequent Washing on the Expected Mortalities of Animals That Were Dermally Exposed to Uranyl Nitrate Hexahydrate in Ethereal Solution*

Detergent	No. of animals	Species	Dose, g/kg	Observed mortality, %	Expected mortality, %	Deg. of freedom	χ^2	p	Percentage of χ^2 contributed by 100% mortality
Soap, 1% solution	5	Rabbit	0.000	0					
Soap, 1% solution	5	Rabbit	0.13	80	52	3	24.16	<0.01	92
Soap, 1% solution	5	Rabbit	0.22	60	67				
Soap, 1% solution	5	Rabbit	0.31	100	76				
$NaHCO_3$, 2% solution	5	Rabbit	0.00	0					
$NaHCO_3$, 2% solution	5	Rabbit	0.13	80	52	3	24.45	<0.01	91
$NaHCO_3$, 2% solution	5	Rabbit	0.22	80	67				
$NaHCO_3$, 2% solution	5	Rabbit	0.31	100	76				
Water	15	Guinea pig	2.0	13	16	1	0.087	0.77	
Cleansing cream	12	Guinea pig	4.6	50	51	1	0.0064	0.94	

*Statistical analysis done by Tiedeman, Kogan, and assistants.

Table 8.10 — Effect of Previous Coating of the Skin on the Expected Mortalities of Animals That Were Dermally Exposed to Uranyl Nitrate Hexahydrate in Ethereal Solution* (One Degree of Freedom in Each Case)

"Protective" coating	No. of animals	Species	Dose, g/kg	Observed mortality, %	Expected mortality, %	χ^2	p
Commercial cream No. 1	8	G. pig	0.00	0			
Commercial cream No. 2 (cocoa-butter cream)	8	G. pig	0.00	0			
Commercial cream No. 1	16	G. pig	2.0	13	16	0.17	0.68
Commercial cream No. 2 (cocoa-butter cream)	15	G. pig	2.0	60	16	9.22	<0.01
Commercial cream No. 3	16	G. pig	4.0	63	45	1.85	0.17
Commercial cream No. 4	16	G. pig	4.0	31	45	1.26	0.26
Commercial cream No. 5	13	G. pig	4.0	69	45	3.05	0.08
Lanolin	16	G. pig	4.0	63	45	1.85	0.17
Mineral oil	15	G. pig	4.0	31	45	1.26	0.26
10% $CuSO_4$	8	Rabbit	0.27	88	73	1.16	0.28
10% $K_3Fe(CN)_6$	8	Rabbit	0.27	50	73	1.51	0.22
5% Picric acid	8	Rabbit	0.27	100	73	38.84	<0.01
10% Tannic acid	8	Rabbit	0.27	100	73	38.84	<0.01

*Statistical analysis was done by Kogan and assistants.

4. METHODS OF LAUNDERING CLOTHES CONTAMINATED WITH URANIUM COMPOUNDS *

Workers' clothing that has become impregnated with one or another compound of uranium is a potential source of dermal exposure. In the case of the soluble compounds of uranium, this would be especially hazardous because of the possibility of elution from the clothing by sweat.

A series of experiments was devised to determine the best conditions for the removal of each of several compounds of uranium from cotton cloth. In the first place, two groups of five 1-ft squares of cotton cloth each were impregnated, respectively, with uranyl nitrate in 5 per cent aqueous solution and uranium ore in 5 per cent aqueous suspension and were then sent to a plant laundry. One additional impregnated cloth from each group was retained for control analysis. Upon the return of the laundered cloths, they were sampled and analyzed. The results indicated that the laundry accomplished nothing in respect to the removal of uranium from the cloth (Table 8.11).

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*Work done by A. B. Carlson and W. F. Neuman.[9]

Secondly, 1-in. squares of cotton cloth were impregnated with a given compound, under the same standard conditions, i.e., by soaking in 5 per cent solutions or suspensions of the compound in question. These pieces were then washed in the laboratory under a variety of conditions and were then analyzed for the amount of uranium retained after washing. Soap and other common washing agents were ineffective in most cases, and in several instances soap appeared to remove less uranium than water alone.

Table 8.11 — Failure of Laundering to Remove Uranium from Contaminated Clothing

		Mg U/sq in.	
Compound	Treatment	Mean	Average deviation
Ore	Laundered	0.78	±0.21
	Nonlaundered	0.56	±0.17
$UO_2(NO_3)_2.6H_2O$	Laundered	3.00	±0.42
	Nonlaundered	2.94	±0.16

Complexing substances, such as bicarbonate, carbonate, citrate, and oxalate ions proved to be by far the most effective agents in removing the soluble hexavalent compounds of uranium (uranyl nitrate, uranyl acetate, uranyl fluoride, and uranyl chloride). From the standpoint of both effectiveness and cost, the most suitable washing agent for general use was a 2 per cent solution of sodium bicarbonate, although each compound presented special problems, and no one general procedure or agent could be recommended for the removal of any and all compounds of uranium. Each of the compounds — uranium tetrachloride, uranium pentachloride, uranium tetrafluoride, uranium peroxide, and uranium trioxide — was satisfactorily removed by a 1 : 1 mixture of 2 per cent hydrogen peroxide and 2 per cent sodium bicarbonate.

In the case of uranyl nitrate, a bicarbonate concentration as low as 0.3 per cent was effective in removing the compound from cloth, and it may be stated that approximately 3 lb of uranium metal (in uranyl nitrate hexahydrate) may be removed by 10 lb of sodium bicarbonate, irrespective of the concentration of the washing solution.

Experiments indicated that the temperature of the washing solution had no consistent effect on the amounts of uranium that could be eluted from cotton cloth. Sodium bicarbonate was effective when mixed with soap, and therefore both uranium compounds and dirt could be removed in one operation.

Although one may conclude that these experimental findings will apply in a general way to all cotton materials, one cannot assume a priori that wool and silk would give the same results.

The direct application of these experimental results to actual laundry practice is not justified, although they do apply in a qualitative way. The development of an actual laundry procedure would have to resolve from the efforts of a group of experimental workers working in close cooperation with a laundry.

From the standpoint of toxicology, bicarbonate or bicarbonate with added hydrogen peroxide rendered contaminated fabric practically free from all toxic compounds of uranium. The compounds of uranium that were not removed by this treatment, i.e., uranium dioxide, uranium tritaoctoxide, and a sample of uranium ore, were not found to be toxic to the skin (see Sec. 1.1c of this chapter).

5. DISCUSSION

It has been shown that certain compounds of uranium will penetrate the intact skin of experimental animals, as evidenced by the presence of detectable amounts of uranium in the blood stream following dermal exposure and by the subsequent development of typical symptoms of uranium poisoning in the exposed animals.

Other heavy-metal salts that are soluble in fats or fat solvents have been shown to penetrate the intact skin via the sebaceous glands. The principles of percutaneous absorption have been reviewed in detail in an excellent paper by Rothman.[10]

The soluble uranyl salts are probably some of the most toxic of all substances that can be absorbed through the skins of experimental animals. Although there is no experimental evidence for the human species, the evidence presented in this chapter indicates that rabbits, rats, guinea pigs, and mice may be fatally poisoned by this route of absorption. The dermal exposure of a small number of dogs[11] also resulted in the typical picture of proteinuria, azotemia, renal pathology, cachexia, and death.

A few of the compounds studied require special consideration. Dosages of uranyl nitrate have been reported, in this chapter, in terms of $UO_2(NO_3)_2.6H_2O$. Although the original form for the compound was the crystalline $UO_2(NO_3)_2.6H_2O$, a large part of the water of crystallization was lost when the solution in ether was made. The recrystallization products on the skin, after the ether had evaporated, probably contained the anhydrous uranyl nitrate, the dihydrate, the hexahydrate, and uranyl nitrate plus ether of crystallization. It is not known which of these forms of the compound actually penetrated the skin. All dosages of the compound therefore have been calculated arbitrarily on the basis of the original form before solution, i.e., the hexahydrate, and have been listed in addition in terms of elemental uranium.

The consensus in the project laboratory is that tetravalent uranium cannot enter the blood stream in appreciable amounts by any route of administration other than parenteral injection (see Chap. 14). The general picture of percutaneous poisoning was one of poisoning from the hexavalent uranyl uranium, and the important factors are those which control the production of uranyl ion, UO_2^{++}, from the various compounds applied to the skin. It is assumed therefore that uranium tetrachloride, which produced typical uranium poisoning following dermal exposure, was oxidized to the hexavalent state before percutaneous absorption took place. On solution of the compound in water, immediate hydrolysis takes place, according to the equation

$$UCl_4 + H_2O \rightarrow UOCl_2 + 2HCl$$

Application of uranium tetrachloride in the form of a dry powder to the skins of experimental animals resulted in rapid solution in the water absorbed from the air, owing to its highly hygroscopic properties. Immediate hydrolysis was thus similarly effected according to the preceding equation.

The uranium oxychloride is subsequently oxidized in the presence of air to uranyl chloride

$$2UOCl_2 + O_2 \rightarrow 2UO_2Cl_2$$

According to experiments reported by Tien Ho Lan,[12] uranium tetrachloride in pure aqueous solution reaches in 30 min an equilibrium containing 80 per cent of the hexavalent uranyl chloride. Conditions on the skin, such as the fatty medium, the pH, etc., may have delayed this oxidation and may thus have caused the slight delay in manifestations of poisoning from uranium tetrachloride, as compared to uranyl nitrate hexahydrate.

A side action, in the form of further hydrolysis of uranium oxychloride to the insoluble and innocuous uranium dioxide

$$UOCl_2 + H_2O \rightarrow UO_2\downarrow + 2HCl$$

may account for the lower toxicity of uranium tetrachloride as compared with the uranyl nitrate hexahydrate.

Uranium pentachloride, in the presence of water, undergoes immediate dismutation with the formation of a mixture of tetravalent and hexavalent salts

$$2UCl_5 + 3H_2O \rightarrow UOCl_2 + UO_2Cl_2 + 6HCl$$

The hexavalent uranyl chloride could, presumably, be absorbed immediately while the uranium oxychloride would first have to undergo oxidation, as shown above. This immediate availability of hexavalent ions may have accounted for the apparently greater proteinuria produced by uranium pentachloride, when applied to the skins of rabbits as a dry powder, than that produced by similar doses of uranium tetrachloride applied in the same manner (see Fig. 8.10).

According to Rothman,[10] percutaneous absorption from fats and fat solvents is usually more rapid and more complete than from water. This seems to have been true for uranyl fluoride and for uranium tetrachloride. The comparative degrees of proteinuria resulting from the dermal application of these two compounds in water and in lanolin were illustrated in Fig. 8.10. On the contrary the mortality of guinea pigs dermally exposed to uranyl nitrate hexahydrate in aqueous solutions was no different from the mortality produced by the same doses of the compound in ethereal solutions.

6. SUMMARY

1. The present investigations have shown that uranyl nitrate, uranyl fluoride, uranium pentachloride, uranium trioxide, sodium diuranate, and ammonium diuranate may be absorbed through the skins of experimental animals to a sufficient extent to cause severe poisoning and death. Uranium has been found in the blood stream of rats following the dermal exposure to uranyl nitrate.

2. Uranium tetrachloride was also found to produce poisoning following application to the skin, but it is believed that oxidation to the hexavalent state is necessary before significant absorption will take place.

3. The insoluble oxides, UO_2, UO_4, and U_3O_8, and the tetrafluoride, UF_4, caused no signs of poisoning following application to the skin.

4. Rabbits, rats, guinea pigs, and mice, in order of decreasing susceptibility, were poisoned by dermal exposure to uranyl nitrate. Species differences were significant, there being more than a hundredfold difference in the LD_{50}'s of rabbits and mice.

5. Although the size of the dose required to produce poisoning varied among species, the general course of poisoning was the same for all species. In the case of rabbits, by the second day following acute exposure, the mean urinary protein was significantly positive, and a few deaths had occurred. On the third day, the mean nonprotein nitrogen of the blood became significantly elevated. On the fifth day, urinary protein had reached a maximum, blood NPN was very high, the mean body weight had dropped significantly, and mortality was highest. The mean NPN reached a maximum on the ninth day, when body weight was also maximally depressed and the mortality was still

relatively high, but the mean urinary protein had fallen to only slightly positive values. Following the tenth day, mean values for all toxicological manifestations began to decrease rapidly and were essentially normal by the eighteenth day following exposure, after which no deaths were observed.

Although blood NPN determinations were not made for the other species, the pattern of urinary protein, body weight, and mortality was essentially the same as for rabbits. The details of minor differences have been indicated in Sec. 1.

6. Renal damage, characteristic of uranium poisoning, was noted microscopically in all the animals that died and were examined. Survivors that had shown signs of severe poisoning, but had recovered, were found to have little histological evidence of renal damage, suggesting a remarkable ability for repair.

7. Repeated dermal exposures resulted in the toleration of cumulative doses that would have been quite lethal if applied as single exposures. Signs of poisoning in the survivors gradually disappeared or lessened after some time, even though the repeated exposures were continued, suggesting an acquired tolerance in survivors.

8. Uranyl nitrate, uranium pentachloride, and uranium tetrachloride produced a mild to moderate, though transient, local irritation of the skin.

9. A number of detergents and "protective coatings" were studied in an attempt to prevent the percutaneous absorption of uranyl nitrate, but none proved to effect any significant protection.

10. Sodium bicarbonate or sodium bicarbonate plus hydrogen peroxide, in aqueous solution, was found to be most effective in removing toxic compounds of uranium from the cotton clothes of workers.

BIBLIOGRAPHY

1. M. DeLaet and C. Meurice, Ing. chim., 8-10: 247 (1924-1926).
2. L. Jaffe, personal communication, Sept. 13, 1945.
3. C. I. Bliss, Quart. J. Pharm. Pharmacol., 2: 196 (1938).
4. C. I. Bliss, Ann. Applied Biol., 22: 307 (1935).
5. F. B. Kingsbury, C. C. Clark, G. Williams, and A. L. Post, J. Lab. Clin. Med., 11: 981 (1926).
6. P. B. Hawk and O. Bergeim, "Practical Physiological Chemistry," p. 418, The Blakiston Company, Philadelphia, 1937.
7. W. F. Neuman, Univ. Rochester, Atomic Energy Project Rept. M-1720 (June, 1945).
8. L. S. Kogan, personal communication, May 6, 1946.
9. W. F. Neuman and A. B. Carlson, Univ. Rochester, Atomic Energy Project Rept. M-1692 (February, 1945).
10. S. Rothman, J. Lab. Clin. Med., 282: 1305 (1943).
11. J. A. Orcutt and M. W. Neuman, Univ. Rochester, Atomic Energy Project Rept. M-1999 (1947).
12. Tien Ho Lan, Univ. Rochester, Atomic Energy Project Rept. M-1668, Sec. III, Part F4 (March, 1945).

Chapter 9

THE TOXICOLOGY OF COMPOUNDS OF URANIUM FOLLOWING APPLICATION TO THE EYE

By James A. Orcutt*

Since the ocular exposure of workers to compounds of uranium, as dusts or in solution, represents a potential hazard and since the ocular exposure of experimental animals in dust chambers represents a possible extraneous route of absorption in the toxicological study of pulmonary exposure, a series of investigations was undertaken to determine the toxicity of a number of these compounds following instillation into the conjunctival sacs of experimental animals. An approximate order of toxicity among the various compounds was determined. A few of the compounds were found to be innocuous in the eye, but others produced moderately severe to severe local lesions, as well as systemic poisoning.

Acute exposures of the eyes of rabbits to uranyl nitrate, uranyl fluoride, uranium trioxide, sodium diuranate, ammonium diuranate, uranium tetrachloride, uranium pentachloride, uranium tetrafluoride, uranium dioxide, uranium peroxide, and uranium tritaoctoxide were studied. Studies of both acute and chronic poisoning from exposure of the eyes of rabbits, rats, and guinea pigs to uranium tetrafluoride were undertaken.

The extreme damage caused by exposure of the eye to minute quantities of certain compounds, solid uranium pentachloride in particular, would suggest that extreme care should be taken to prevent industrial ocular exposure to these compounds.

Since the data of this chapter were assembled from isolated experiments or groups of experiments, each designed for a special purpose of its own, the various compounds were not in all cases tested under identical conditions with respect to vehicle and concentration. Not all the data concerning these compounds, therefore, are directly comparable. The seemingly far greater damage caused by uranium pentachloride than by any of the other water-soluble compounds may have been due to the fact that it was applied in dry form. Thus, perhaps uranium tetrachloride, uranyl nitrate, or uranyl fluoride, if applied in the solid form, might be just as destructive as uranium pentachloride.

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*Work done by James A. Orcutt, Raphael E. Maiers, and Nancy B. Smith.

Most of the rabbits employed in these experiments were New Zealand Whites and, in addition, there were Chinchillas, Checkers, and New Zealand Reds. Animals of both sexes were used, and the average weight was slightly over 3 kg.

Guinea pigs and rats were used only in the studies of acute and chronic poisoning from uranium tetrafluoride. The guinea pigs were males and females ranging in weight from 500 to 700 g. The rats were male albinos weighing approximately 300 g.

1. ACUTE POISONING

A single large dose of each compound was instilled into the conjunctival sac, in the form of a dry powder, an aqueous solution, a suspension in lanolin, or a suspension in water.

For the suspension in lanolin, the compound was added to an equal weight of lanolin and mixed thoroughly with a spatula. This resulted in an approximately 50 per cent suspension. Adequate contact of the material with the cornea was ensured in every application. Clinical observations were made, with the assistance of D. E. Bacon, D.V.M., on the first 4 to 10 days following exposure, according to the severity of the initial reaction, and twice a week thereafter until death or complete recovery had occurred.

The degrees of conjunctival inflammation, edema, exudation, and ulceration were recorded as 1+ to 4+; and the degrees of corneal cloudiness, corrosion, ulceration, and vascularization were similarly recorded. Corneal corrosion was detected by applying a 2 per cent aqueous solution of fluorescein to the cornea, removing the excess by flushing with distilled water or physiological saline, and examining the cornea for spots of fluorescence with the aid of a flashlight.

Dates of death were recorded, and the percentage mortalities were calculated. The experimental results have been presented in Table 9.1. From these observations it is possible to make a tentative classification of the various compounds according to relative local damage and systemic toxicity in rabbits (see Table 9.2).

Uranyl nitrate, uranyl fluoride, uranium tetrachloride, uranium pentachloride, uranium trioxide, sodium diuranate, ammonium diuranate, and uranium tetrafluoride were absorbed from the conjunctival sac in sufficient quantities to produce severe systemic poisoning and death of some animals. Dosages of each of these compounds (with the exception of uranium trioxide) were, fortuitously, intermediate in size, just large enough to kill 25 to 75 per cent of the animals and to allow one or more survivors for protracted observations.

1.1 Uranium Pentachloride. One milligram of this compound placed in the conjunctival sac of each of four rabbits produced death, from systemic poisoning, of two of the animals and severe, irreparable damage to the eyes of all four animals, leading eventually to necrosis and separation of conjunctivas, eyelids, and periorbital tissues and peeling and ulceration of the corneas with perforation into the anterior chambers.

The severe local action of uranium pentachloride was probably due to the caustic action of the very high local concentrations of hydrogen chloride liberated on hydrolysis of the compound. From every milligram of uranium pentachloride, 0.26 mg of anhydrous HCl would theorectically be liberated according to the equation

$$2UCl_5 + 3H_2O \rightarrow UOCl_2 + UO_2Cl_2 + 6HCl$$

1.2 Uranium Tetrachloride. This compound undergoes immediate hydrolysis in the presence of water, according to the equation

$$UCl_4 + H_2O \rightarrow UOCl_2 + 2HCl$$

The uranium oxychloride so formed then undergoes oxidation in the presence of air according to the equation

$$2UOCl_2 + O_2 \rightarrow 2UO_2Cl_2$$

So-called "aqueous solutions of uranium tetrachloride" therefore were actually solutions of a mixture of uranium oxychloride and uranyl chloride. These solutions, when applied to the eye, produced systemic poisoning and severe corneal and conjunctival damage, which, in the single survivor, had shown no tendencies toward healing by the 48th day following exposure.

1.3 Uranyl Nitrate and Uranyl Fluoride. The aqueous solutions of these hexavalent compounds, when instilled into the conjunctival sac, produced systemic poisoning and severe corneal and conjunctival damage. Survivors from exposure to either of these compounds showed complete ocular recovery by the 28th day following exposure.

1.4 Sodium Diuranate. After instillation of dry powdered sodium diuranate into the conjunctival sac, systemic poisoning was observed as well as severe corneal and conjunctival damage. Recovery from

Table 9.1 — Toxic Manifestations Following Applications of Various Compounds of Uranium to the Eye

Expt. No.	Compound	Conc., %	Vehicle	Species	No. of animals	Grams per eye	Exposed eye	Observed mortality, %
Single Exposures								
1	UCl_5	100	None	Rabbit	4	0.001	Right	50
2	UCl_4	50	Water	Rabbit	4	0.25	Right	75
3	UO_2F_2	40	Water	Rabbit	4	0.20	Right	75
4	$UO_2(NO_3)_2 \cdot 6H_2O$	70	Water	Rabbit	4	0.35	Right	75
5	$Na_2U_2O_7$	100	None	Rabbit	4	0.10	Right	25
6	$(NH_4)_2U_2O_7$	100	None	Rabbit	4	0.10	Right	25
7	UF_4	100	None	Rabbit	6	0.10	Right	50
8	Talc	100	None	Rabbit	6	0.10	Left	0
9	UO_2	50	Lanolin	Rabbit	4	0.75	Right	0
10	UO_2	100	None	Rabbit	4	0.10	Left	0
11	UO_3	50	Lanolin	Rabbit	4	0.75	Right	0
12	UO_3	100	None	Rabbit	4	0.10	Right	100
13	UO_4	50	Lanolin	Rabbit	4	0.75	Right	0
14	UO_4	100	None	Rabbit	4	0.10	Left	0
15	UO_4	5	Water	Rabbit	4	0.025	Right	0
16	U_3O_8	50	Lanolin	Rabbit	4	0.75	Right	0
17	U_3O_8	100	None	Rabbit	4	0.10	Left	0
18	Talc	100	None	Rabbit	2	0.10	Both	0
19	UF_4	100	None	Guinea pig	6	0.01	Right	0
20	Talc	100	None	Guinea pig	6	0.01	Left	0
21	UF_4	100	None	Rat	6	0.01	Right	0
22	Talc	100	None	Rat	6	0.01	Left	0
23	Talc	100	None	Guinea pig	1	0.01	Both	0
24	Talc	100	None	Rat	2	0.01	Both	0
Repeated Exposures								
25	UF_4	100	None	Rabbit	6	0.10	Right or left	100
26	UF_4	100	None	Guinea pig	5	0.01	Right	0
27	UF_4	100	None	Rat	6	0.01	Right	0

Table 9.1 (Continued)

	Evaluations of maximal damage attained during experiment							Day of complete recovery for survivors
	Conjunctivas				Corneas			
Expt. No.	Inflammation	Edema	Exudation	Ulceration	Cloudiness	Corrosion	Vascularization	
				Single Exposures				
1	4+	4+	4+	4+	4+	4+	2+	*
2	2+ to 4+	2+ to 3+	1+ to 3+	0	0 to 2+	0 to 1+	0 to 2+	†
3	2+	1+ to 2+	1+ to 2+	0	0	0 to 3+	0	28th
4	3+ to 4+	0 to 2+	4+	0	2+ to 4+	3+ to 4+	0 to 3+	26th
5	2+ to 4+	0	2+ to 3+	0	1+ to 2+	1+ to 4+	0	9th
6	3+	0	0 to 1+	0	0	0	0	6th
7	0 to 1+	0	0 to 1+	0 to 1+	0 to 1+	0	0	‡
8	1+	0	0	0	0	0	0	
9	0 to 1+	0	0	0	0	0	0	4th
10	0 to 1+	0	0	0	0	0	0	2nd
11	0 to 1+	0	0	0	0	0	0	4th
12	0 to 1+	0	0	0	0	0	0	2nd
13	0 to 1+	0	0	0	0	0	0	3rd
14	0 to 1+	0	0	0	0	0	0	2nd
15	0	0	0	0	0	0	0	
16	1+	0	0	0	0	0	0	3rd
17	0	0	0	0	0	0	0	
18	0 to 1+	0	0	0	0	0	0	6th
19	0	0	0	0	0	0	0	
20	0	0	0	0	0	0	0	
21	0 to 1+	0	0	0	0	0	0	9th
22	0 to 1+	0	0	0	0	0	0	2nd
23	0	0	0	0	0	0	0	
24	0 to 1+	0	0	0	0	0	0	2nd
				Repeated Exposures				
25	2+ to 4+	0	1+ to 4+	1+ to 4+	0 to 3+	1+ to 3+	0 to 2+	§
26	0 to 1+	0	0	0	0	0 to 1+	0	
27	1+	0	0	0	0	0	0	10th

*No remission in survivors by the 45th day; also necrosis of conjunctivas and eyelids and peeling and ulceration of the corneas with perforation into the anterior chamber; maximal values for urinary protein ranged from 150 to 280 mg/100 ml.

†Single survivor had not recovered on the 48th day.

‡Sacrificed on the 6th day; maximal values for urinary protein ranged from 30 to 150 mg/100 ml.

§Also 0 to 3+ ulceration of the corneas, without perforation; maximal values for urinary protein ranged from 80 to 400 mg/100 ml.

Table 9.2—Approximate Order of Toxicity of Various Compounds of Uranium When Instilled into the Conjunctival Sacs of Rabbits

Compound	Vehicle (if any)	Degree of local ocular damage	Time of recovery	Systemic effect
UCl_5	Dry powder	Severe, with necrosis and perforation	Not by 7th week	Poisoning
UCl_4, $UOCl_2$	Water	Severe	Not by 7th week	Poisoning
UO_2F_2	Water	Severe	4 weeks	Poisoning
$UO_2(NO_3)_2.6H_2O$	Water	Severe	4 weeks	Poisoning
$Na_2U_2O_7$	Dry powder	Severe	2 to 9 days	Poisoning
UF_4	Dry powder	Moderate	2 to 9 days	Poisoning
$(NH_4)_2U_2O_7$	Dry powder	Moderate	2 to 9 days	Poisoning
UO_3	Dry powder	Mild irritation only	2 to 9 days	Poisoning
UO_3	Lanolin	Mild irritation only	2 to 9 days	None
Talc	Dry powder	Mild irritation only	2 to 9 days	None
UO_2	Lanolin	Mild irritation only	2 to 9 days	None
UO_2	Dry powder	Mild irritation only	2 to 9 days	None
UO_4	Lanolin	Mild irritation only	2 to 9 days	None
UO_4	Dry powder	Mild irritation only	2 to 9 days	None
U_3O_8	Lanolin	Mild irritation only	2 to 9 days	None
U_3O_8	Dry powder	None		None

this local ocular damage, however, was very rapid. There was no visible remnant of the damage in any of the survivors by the 9th day following exposure.

1.5 Uranium Tetrafluoride. This compound was similar in effect to sodium diuranate, except that the local ocular damage was somewhat less severe. The animals were sacrificed before complete recovery had occurred.

Since the toxicity of the theoretically insoluble uranium tetrafluoride may have been due to impurities, in the form of soluble hydrolysis products present in the sample, its content of soluble uranium was determined in the following manner: 1 g of the sample of uranium tetrafluoride was shaken with 9 ml of water and then centrifuged. The supernatant liquid had a pH of 4.0 and contained 0.015 g of uranium calculated as uranyl fluoride. This corresponded to 1.5 per cent of uranyl fluoride in the original sample of dry powdered uranium tetrafluoride.

1.6 Ammonium Diuranate. This compound produced systemic poisoning but only moderate conjunctival irritation of no more than 6 days' duration in the survivors and no damage to the cornea.

1.7 Uranium Trioxide. This compound, applied as a dry powder, was unique in that it caused no significant local effect on the eye but was absorbed systemically to a sufficient extent to produce death in all of four animals studied. The incorporation of uranium trioxide in lanolin, however, apparently prevented its conjunctival absorption, as evidenced by the lack of symptoms of poisoning even when 7½ times the dose was instilled into the conjunctival sac in suspension in lanolin.

1.8 Uranium Dioxide, Uranium Peroxide, and Uranium Tritaoctoxide. None of these compounds caused any evidence of systemic poisoning or any local effect more than a mild mechanical irritation similar to that seen after the instillation of powdered talc into the conjunctival sac. Dry powdered uranium tritaoctoxide caused no visible damage, not even the mild mechanical irritation seen after exposure to powdered talc. This compound, therefore, serves as a good control for the other compounds of uranium in the experimental study of ocular toxicology.

2. CHRONIC POISONING

Chronic studies were undertaken for uranium tetrafluoride only. The dry powder was placed in the conjunctival sac on each of 10 successive days.

The degrees of conjunctival inflammation, edema, exudation, and ulceration, as in Sec. 1, were recorded as 1+ to 4+; and the degrees of corneal cloudiness, corrosion, ulceration, and vascularization were similarly recorded.

Dates of death were recorded, and the percentage mortalities were calculated.

The rabbits that were exposed to uranium tetrafluoride were kept in individual metabolism cages, and a few 24-hr samples of urine were collected. They were tested for protein by the method of Kingsbury, Clark, *et al.*[1] and for pH.

The experimental results of studies of the effects of repeated exposures of the eye to uranium tetrafluoride have been listed in Table 9.1.

The toxicity of this compound may have been due to its original content of uranyl fluoride or to its further hydrolysis and oxidation to uranyl fluoride within the conjunctival sac. In the presence of water and air, the following reactions take place very slowly because of the low solubility of uranium tetrafluoride:

$$UF_4 + H_2O \rightarrow UOF_2 + H_2F_2$$

$$2UOF_2 + O_2 \rightarrow 2UO_2F_2$$

In any case, local damage and systemic poisoning from uranium tetrafluoride were observed only in rabbits. The mild irritation observed upon application of the compound to the eyes of rats and guinea pigs was no greater than that produced in the opposite eyes of the same animals by powdered talc.

Aside from the fact that rats and guinea pigs are by nature less susceptible to uranium poisoning, the rats were observed to rub their eyes industriously immediately after exposure to the compound, and, on the day following exposure, no traces of it could be found in the conjunctival sacs. Though the guinea pigs were not observed to rub their eyes, the compound had similarly disappeared on the day following each exposure. Large quantities of the compound, however, were found adhering to the corneas and conjunctivas of the rabbits 24 hr after it had been instilled into the conjunctival sacs. This may serve to illustrate the importance of washing and removing all particles of uranium compounds from accidentally exposed eyes.

The single applications of uranium tetrafluoride to the eyes of rabbits produced only moderate local damage as compared with other compounds of uranium (see Table 9.1). But repeated applications (0.1 g per eye per day for 10 days) resulted in local damage as severe as that produced by any of the most toxic compounds (see Fig. 9.1), with the exception of dry powdered uranium pentachloride, and resulted in the death of all the animals so exposed.

Although the damage caused by uranium tetrafluoride was considerably more severe than that caused by similar quantities of powdered talc or of uranium tritaoctoxide, it is still possible that this local damage may have been due to repeated mechanical irritation and foreign-body reaction. The danger from systemic absorption after exposure of the eye to relatively large quantities of uranium tetrafluoride, however, is apparent. This systemic toxicity is presumably due to the previously-mentioned decomposition products of uranium tetrafluoride.

BIBLIOGRAPHY

1. F. B. Kingsbury, C. C. Clark, G. Williams, and A. L. Post, J. Lab. Clin. Med., 11: 981 (1926).

The substance of this chapter was taken from the following reports:

J. A. Orcutt and N. B. Smith, Univ. Rochester, Atomic Energy Project Rept. M-1710 (Mar. 21, 1945).

J. A. Orcutt, Univ. Rochester, Atomic Energy Project Rept. M-1687, Sec. III, Part E, 7 (Apr. 25, 1945).

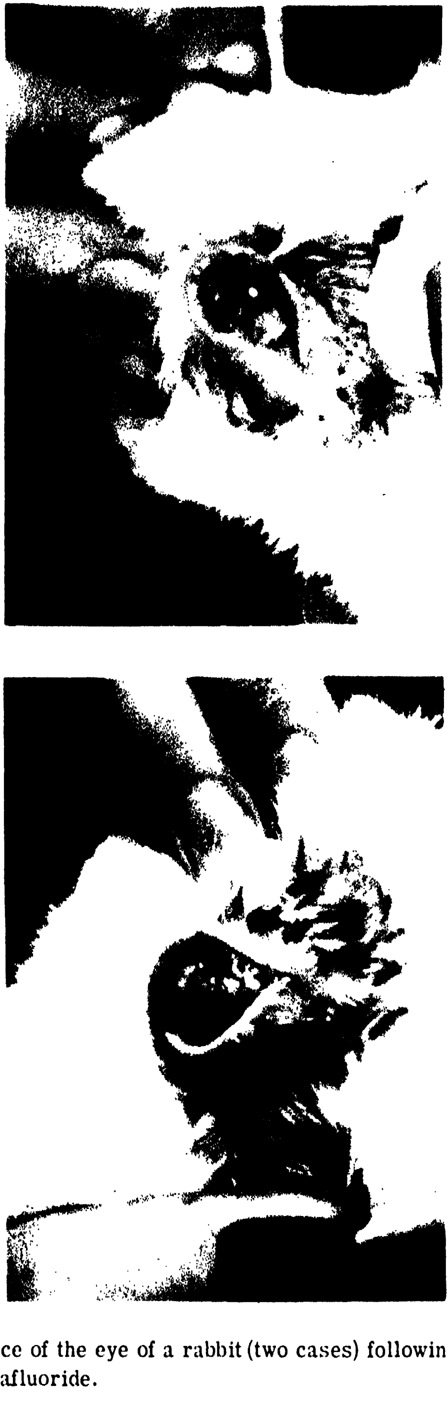

Fig. 9.1 — Appearance of the eye of a rabbit (two cases) following repeated daily exposure to uranium tetrafluoride.

Chapter 10

TOXICITY FOLLOWING INHALATION

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PART A. INTRODUCTION

The purpose of this account is to summarize the toxicologic data obtained from exposure of animals to the inhalation of uranium dusts. Specific recommendations for some simple measures of protection for the worker against dust hazards, based on results of tests of respiratory protective devices, are also presented. The information is presented in some technical detail and is intended to be of service to toxicologists, medicolegal practitioners, plant supervisors, industrial hygienists, plant physicians, and technicians and engineers concerned with the safety of personnel who handle uranium dusts.

The chapter is broadly divided into introduction (Part A), procedures (Part B), presentation of results of short-term exposure studies (Part C), results of respirator tests (Part D), and a discussion of the significance of the results (Part E).

Because of the extensive nature of the work, complete data of all experiments cannot be included under the various experiments. Hence certain characteristic results of the toxicologic response by inhalation have been selected in the following reports.

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* Work done under the direction of Dr. H. E. Stokinger by E. Roberts, A. Rothstein, C. J. Spiegl, and B. H. Amdur, R. C. Baxter, H. L. Berke, C. W. Bishop, J. Brinkman, D. C. Brodie, J. Cobler, D. Dittman, H. P. Dygert, N. Eriksen, J. B. Field, A. Fiorica, R. Gerson, W.M. Harrison, C. Horton, C. W. LaBelle, S. Laskin, G. Laush, R. Maiers, J. Minor, H. A. Oberg, A. G. Pettengill, U. C. Pozzani, Martha Reid, J. J. Rothermel, E. Same, R. Sanford, A. I. Schepartz, M. Schlamowitz, A. I. Shannon, G. F. Sprague, Jr., J. A. Tornaben, C. S. Weil, F. W. Wichser, H. Wills, and H. Wilson.

1. STATEMENT OF PROBLEM

The studies were begun at the request of the Medical Section of the Manhattan District Project. They were intended to provide information from which safety standards could be developed for the control of health hazards resulting from the contamination of factory air with uranium dusts arising in the course of manufacture of the products related to the atomic bomb. The development of such standards was to be based upon knowledge obtained from both short- and long-term exposures of various animal species to atmospheres containing different concentrations of the industrially important uranium dusts. The assumption was that such knowledge could be related in a general way to problems of human exposure. Information on chemical toxicity alone was to be sought. The radioactivity of the uranium materials is known to be low, and when, by calculation, an approximation was made of the amounts of uranium inhaled, it appeared that the possibility of toxicity from radiation would be remote. The testing of respiratory protective devices for the use of plant personnel was a further assignment. A derivative development was the provision of techniques and personnel for collaborative tests to assist plant medical supervisors. Thus, the aim of the entire project was to eliminate, in so far as possible, injury to the health of workers subjected either occasionally or regularly to exposure to dusty atmospheres of uranium-containing materials, at concentrations accidentally high or uniformly low.

1.1 Background. In the literature on inhalation no record exists of exposures of animals to uranium dusts. In fact, there is a relative paucity of pertinent reports on experimental dust exposure. A certain amount of work with experimental animals has been done on nonuranium dust in mines.[1] A number of dusts have been investigated in reference to their production of respiratory disorders in animals. The dusts studied at various sites and in laboratory tests have included those of coal, shale, flint, granite,[2] marble,[3] asbestos,[4] carborundum,[5] silica, lead, and metallic oxides.[6] Methods have been established by the U. S. Bureau of Mines for testing of respirators,[7] and a number of respirators have been certified for use in dusty atmospheres containing these materials.

One of the earliest records of dust-inhalation exposures of animals appeared in a monograph by Arnold[8] in 1885. Because this experimenter was primarily interested in the origin of a melanin-like pigment in the lung, the study revolved about the possible role of retention in the lung of different types of comparatively nontoxic dusts such as carbon black, ultramarine, emery, and sandstone. For this reason the channels of entry of dust into the body and its transportation, retention, and pathology were emphasized, but little attention was paid to dust control and none to the quantitative measurement of dust in the inhaled air, factors of prime concern in the present program. A similar criticism may be made against a prior dust-inhalation study by von Jns.[9]

One of the earliest quantitative studies of dust exposures of animals was by Saito,[10] working on lead compounds in Lehmann's laboratory in Wuerzburg in 1912. Relatively elaborate methods were devised for exposing the animals and the measurement of the dust concentration, but no controlled method of introducing the dust was described. Only the heads of dogs and rabbits were subjected to dusts of white lead oxide for periods of from 1 to 33 hr under known conditions of air flow and dust concentration. Concentrations were of the order of 300 mg/cu m, approximately tenfold greater than the highest concentrations of uranium compounds employed in the work at Rochester. Animal tissues were analyzed for their lead content.

Another early experiment on the effect of dust inhalation by animals was that of Mavrogordato[11] in Oxford, England, in 1918. Oddly, the methods of dust production and measurement were cruder than in the earlier work. Dust clouds for inhalation were produced by having a fan blow air upon a box containing the finely ground materials, i.e., coal, shale, flue dust, and flint. Enormous dust concentrations ranging from 27,000 to 45,000 mg/cu m were employed. Shortly after this report, Gardner[12] published results of effects of exposures of guinea pigs to granite dust in relation to susceptibility to tuberculosis. This report also contains a number of references to earlier studies. A rough method of producing dusty atmospheres is given, but no measurement of concentration was made. Particle sizes ranged from 0.28 to 84 μ. Exposure of animals was from 6 to 8 hr daily and continued from 2 to 7 months.

These early reports dealing with relatively nontoxic dusts, although serving the purposes of the investigators who were primarily interested in animal response, were of little aid in the present program. Here highly toxic dusts were used, and controlled methods of dust feeding and accurate measurement of dust concentration were demanded.

More recently, however, Fairhall and collaborators[13] have made important advances in the field of dust exposures, chiefly through the use of improved methods for the production of dusty atmospheres and their accurate estimation. These workers have been mainly interested in the toxic effects produced from the exposure of animals to heavy-metal dusts, e.g., lead, arsenic, and antimony. The method of Fairhall for the estimation of dust concentration has been used with only slight modifications throughout the work reported here.

Although a number of devices for the production of dusty atmospheres are described in the literature, none were found that were suitable for use with uranium dusts. Therefore the models used in the present studies were devised by the engineering group to fit the particular requirements of high density, fine-particle-size dusts.

The methods used for testing respirators were those recommended by the U. S. Bureau of Mines.[18] The only important difference in our procedure was the replacement of the prescribed electrostatic precipitator by the filter-paper sampler of Fairhall[13] for the determination of the dust that penetrated the respirator filters.

1.2 Interim Choice of Maximal Allowable Dust Concentration. For the certification of respirators a limit prescribing the maximal amount of dust that could pass through the filters was obviously needed. A permissible limit of exposure was also required for engineers as a guide in controlling the amounts of uranium dust in factory air. With only the toxicologic information on uranium by the parenteral route available, which suggested a toxicity for uranium of the same order as that of lead, a figure of 150 μg of uranium metal per cubic meter of air was tentatively selected. This concentration had been the permissible limit set for exposures to lead and, assuming the inspiration of 10 cu m of air, predicates an intake of not more than 1.5 mg of metal during an average working day. This choice for uranium was felt to be both reasonable and conservative and in addition had the advantage of the demonstration by the lead industry that dust in factory air can be maintained within this limit.

The uranium in the compounds under consideration in this report refers to the naturally occurring isotopic mixture. Uranium as it occurs in nature contains three isotopes, all spontaneously radioactive. These are U 234, U 235, and U 238, in amounts of approximately 0.006, 0.7, and 99.3 per cent, respectively. The major component U 238, has a radioactivity of approximately 1/2,000,000 that of radium; U 234 has a radioactivity of about 2,300 times that of U 238; and U 235 has approximately ten times the radioactivity of U 238.

2. METHOD OF ATTACK OF PROBLEM

2.1 Basic Procedures. Two basic procedures of toxicology were used for the inhalation studies, namely,

1. Determination of the acute effects of exposure of animals. A 30-day test period was arbitrarily chosen in order to provide a maximal amount of information in the minimal time demanded by the urgent wartime requirements. This later turned out to be a most

fortunate choice because in practically every instance the effects of acute exposure to uranium became manifest within the first week of exposure and produced the mortality that followed by the end of the third week.

2. Prolonged exposure studies, of 1 year's duration.* The short-term study provides a rapid evaluation of the approximate toxicity of a given uranium dust, a "pilot" test showing the order of toxicity; the knowledge thus obtained serves as a guide to the design of the long-term exposures. Information derived from prolonged exposures allows in turn the precise determination of a permissible level of exposure for animals from which no toxic manifestation might be expected regardless of the duration of the exposure. Prolonged exposures provide also an answer to the question as to whether uranium produces the entity "chronic intoxication."

2.2 Organization of Inhalation Program. (a) Exposure Units. The inhalation program required the construction of several large chambers for the exposure of large numbers of animals of the common laboratory species. Chambers of various design were constructed because it was not known at the start of the project which design would prove the most suitable type for exposure of animals to uranium dusts; precedents for large-scale dust-exposure experiments were few. Although construction was begun on only a few units, the number of chambers was ultimately enlarged to 10 units. These have been in operation for approximately 2 years.

(b) Number of Studies, Materials, Levels. The expansion of the Inhalation Section occurred rapidly as the extent of the program was recognized. Information on the toxicity of a minimum of 12 uranium dusts was essential. Because it was realized that the time required to obtain the necessary information by inhalation procedures would be protracted, the uranium materials were placed on a priority list; the position on the list was based on the extent of the exposure hazard and the adjudged toxicity of the material. These priorities were reviewed from time to time as toxicologic information increased. The number of concentration levels at which the dusts were to be tested depended on these priorities. For materials of high priority, from four to seven different levels were set for study; from one to three levels were selected for the compounds of lower priority rating. With these ratings as a basis, 46 thirty-day exposure studies (see Part II for other studies of nonuranium materials, made concurrently), of which 5 were controls, were completed; and 13 prolonged-exposure studies, one of which was a control, were undertaken.

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* The four terms (acute, chronic, short-term, and prolonged exposure) used in this monograph are defined in the following manner: Acute exposure refers to a type of response, rapid in onset with extensive and severe changes frequently resulting in death; chronic exposure, a response of persistent or recurrent changes, generally milder in nature, which may or may not terminate in death; short-term exposure refers to duration of exposure, repeated daily exposures of 6 hr each for a period of 30 exposure days or less; prolonged exposure, daily repeated exposure for a period of 1 year.

(c) Personnel. The personnel working with each animal-exposure chamber was organized as a unit and was headed by a biochemist with a Ph.D. degree or its equivalent. The unit head was aided by from three to five assistants of M.S. or B.S. training. The duties of the unit head consisted of organizing and planning the studies, the general supervision of the unit, and the writing of reports. In addition the unit heads were charged with the development of special tests relating to problems directly associated with the work of their particular unit. The assistants, as well as the unit heads, were responsible for the handling of the animals, loading the units, obtaining specimens for biochemical tests, control and operation of the exposure units, and aid in the development of special tests.

(d) Collaborative Departments. In order to ensure the maximal possible information from either the short- or long-term studies, a well-integrated group of accessory departments comprising different branches of the medical and chemical sciences was organized. These embraced the departments of pathology, surgery, biochemistry, physiology, hematology, and analytical chemistry, each with trained and competent staffs, as well as other units organized to supply special types of data. The latter included a group of experts on determination of particle size of dust and another on development of special methods relating to the problems of dust retention in the animal organism. In addition, an engineering division was organized to meet the difficult problem of constructing exposure chambers, to design and develop dust feeds, a little-investigated subject, to air-conditioning the units, and to handle related problems. At the outset a veterinary service was established to procure animals suitable for the experiment and to maintain their health. A small group was placed in charge of assigning and distributing animals to the various units, and a group of animal caretakers was assigned to care for the animals under the supervision of a foreman of long experience. Apart from these required facilities, the need for accessory and new biochemical tests of toxicity arose during the early stages of the work. Such needs were supplied by a capable group of biochemists attached to the individual exposure units who developed new and extremely sensitive indicators of early uranium poisoning. Certain of these tests later found application in evaluating toxic exposures in plant personnel (for specific tests, see Chap. 5). A large statistical department cooperated in evaluating the results of toxicologic data.

Special procedures employed by the various departments, either procedures developed expressly for this section or procedures that aided greatly in evaluation of toxicologic response, are mentioned briefly in what follows:

(1) Pathology. A procedure of serial sacrifice of animals for histologic study was used, chiefly for dogs and rats on either short-term or prolonged studies, wherein animals were sacrificed either singly or in groups periodically throughout the course of the experiment in order to allow the progress of toxicity to be followed.

(2) Surgery. A procedure of serial renal (and in one case also hepatic) biopsies of dogs permitted the course of damage to be evaluated in a single animal at critical periods during exposure.

(3) Biochemistry and Physiology. All tests described in Chap. 5 were applied to animals of this section.

(4) Hematology. Twenty variable elements of blood were studied at evenly spaced periodic intervals before and during exposure of the animals.

(5) Analytical Chemistry. The Inhalation Section used this branch of science chiefly for routine analysis as follows:

1. The concentration of uranium dust in the exposure chambers by the chemical method employing ferrocyanide (Chap. 2).
2. The content of uranium and lead in animal tissues by the spectrographic method (Chap. 2).
3. The content of uranium in animal tissues by the fluorophotometric method (Chap. 2).
4. The content of fluoride ion in animal tissues by a modification of the chemical method of Willard and Winter (Chap. 2).

(e) Over-all Number of Animals. The entire 3-year inhalation program entailed the use of somewhat more than 12,000 (including those animals used in experiments summarized in Part II on fluorine compounds) laboratory animals of six species; for the acute studies of uranium dusts, 2,600 rats, 160 dogs, 780 rabbits, 1,200 guinea pigs, 2,500 mice, 60 cats, and 100 hamsters; for the 13 chronic studies, 3,000 rats, 250 dogs, 130 rabbits, and 175 guinea pigs; and for the control studies, 600 rats, 50 dogs, 250 rabbits, and 150 guinea pigs.

3. HISTORY OF PROGRESS AND DEVELOPMENT

The story of the development of the Inhalation Section is typical of wartime research projects. In January 1944, during the early days of the project, buildings, equipment, and personnel could not keep pace with the expanding horizons of fast-developing and urgent health problems. Although this brief history records only the progress and development of the inhalation studies dealing with uranium dusts, the Inhalation Section was assigned two additional major projects: (1) that of determining the toxicity of fluorine and certain of its gaseous derivatives on laboratory animals as well as (2) the responsibility of obtaining similar information on special materials · related to the technology of uranium. The latter required large-scale and accurate testing of certain of the more important materials and involved personnel and equipment requisite for handling on short notice small-scale pilot testing of certain compounds of special industrial importance. Information on the latter tests was desired within 24 to 48 hr, and emphasizes the stress that was placed on the personnel and equipment at the start of the program. The results of these studies are described separately in Part II of this volume.

Soon after the start of the inhalation work it became apparent that the existing space set aside for the exposure chambers, animal quarters, and laboratories was insufficient to handle the problems submitted by the district medical officer; therefore a wing was added that tripled the floor space originally assigned to this section. At this time (December 1943) the personnel of this section consisted of 23 scientists, including 8 engineers who were engaged in the design of toxicological experiments, design of exposure chambers and dust feeds, and plans of future expansion. At this time only two 30-day studies (rather crude as judged by subsequent results) had been completed. By June 1944 the group had been expanded to 38 scientific workers and 8 engineers; eight 30-day studies had been completed. Difficulties of procuring scientifically trained personnel, of acquiring large numbers of animals, of constructing and of obtaining equipment, despite highest priorities, were still hampering research production to such an extent that by January 1945, although the technical staff had been expanded to 53, only 19 of the ultimate 46 thirty-day studies had been completed, and certification of respirators had been made for 10 of the uranium dusts. Furthermore, toxicity information had been gathered for distribution to the district on but one concentration level of most of the 12 dusts.

A critical situation had been reached. Regrettably few acute exposures had been completed, and no chronic studies had been started. It was the latter studies that ultimately were expected to be of chief practical value. Obviously the formation of a new policy of procedure was necessary. Otherwise important data would be gathered too late.

Joint planning sessions were held with the district medical officers and the heads of the local research group for the formulation of an experimental policy to expedite the acquiring of desired toxicity data. A number of plans involving various combinations of acute and chronic exposure studies were possible. Suggestions were made to abandon all short-term exposures and immediately start prolonged exposures. In the latter instance two points of view were favored, bearing in mind the limitations set by the number of exposure units: (1) to study one-half dozen levels each of two of the most important uranium dusts and (2) alternately to study one level of each of twelve of the uranium materials. The former possessed the advantage of accurate delineation of the toxicity of two dusts, the latter, the advantage of offering scope to the toxicity survey. Eventually the compromise was adopted of greatly curtailing the number of 30-day studies. Only those which would supply much-needed information for the study of five dusts of high priority rating were to be continued. Two levels for each of the five dusts were demanded in order that a minimum of data be obtained that would be sufficiently reliable to set limits for safe human exposure, the chief goal of the prolonged toxicity studies. With the equipment at hand, and with the aid of small numbers of additional personnel, this program could be accomplished only by putting the entire inhalation program on a two-shift daily basis, with the occasional demand of a three-shift day. The Inhalation Section functioned under this arrangement from March 1945 until the completion of the work in September 1946.

3.1 Planning Conferences. The very worth-while procedure of holding planning sessions for each exposure study was resorted to at the outset of the work. In attendance at the conference were the unit head and his assistants who were to perform the experiment. Also present were the head of the Pharmacology Division and the heads of all other divisions who were concerned with the study. Prior to the meeting a detailed memorandum was circulated in which the purpose and the proposed manner of executing the experiment was outlined. At the meeting, the organization of the experiment, coordination of the work of collaborating divisions, the number of animals, type of data to be obtained, etc., were determined. A final report of this meeting, containing any changes or addenda, was subsequently circulated to all those involved in the study. This planning-session report served as a guide to the experimenters and as a permanent record of the type of work performed. As a result of this policy it was rare that any change of significance was required subsequently in the experimental procedure.

4. VALUE OF INHALATION STUDIES TO HEALTH PROBLEM

From the viewpoint of industrial hygiene, the determination of the toxicity of uranium dusts by inhalation yields more useful information than that from other experimental routes of absorption because the respiratory tract is the chief route by which uranium enters the body in the course of daily work. Consequently the results of inhalation-exposure studies most closely simulate actual exposure conditions among plant personnel. A certain amount of uranium material enters the body by ingestion, still smaller amounts enter through cuts and abrasions of the skin, and there is a possibility of some entering through the eye. But each of these other routes of entry constitutes a far smaller problem. A certain additional advantage of the experimental inhalation procedure is the ability to reproduce actual plant conditions. The dusts used for the experiments were taken directly from plant operations, and the range of concentrations and particle size of dusts encountered in factory air were approximated in these experimental procedures.

On the other hand there are certain disadvantages inherent in the inhalation procedure that cannot be obviated. Inhalation experiments are complicated by the uncertainty of the amount of dust received by the animal. Two factors contribute to this. The first of these is the inability to maintain a concentration of dust at a definite value; the concentration to which the animals are exposed is more often a range rather than a precise value owing to the numerous difficulties associated with introduction of dusts into the exposure chamber, the shortcomings of dust feeds, and the maintenance of uniform distribution of dust within the chambers. Accordingly the dosage of uranium that an animal receives by inhalation is not (and cannot be) accurately known. The second factor is the differences among species, such as differences in respiratory tracts and differences in breathing and sleeping characteristics. Smaller species have a higher respiratory rate than larger species; certain species such as the rat and mouse sleep a considerable part of the day, not at night; the dog sleeps during part of the exposure time, the guinea pig and rabbit infrequently. The differences in the lining of the respiratory tract of different species occasion in one species the unavoidable entry of more dust into the gastrointestinal passages than in another. The amount of licking of the fur varies among species. All these considerations introduce certain complications in the interpretation of the results on a strict inhalation basis. Absorption of certain dusts through the eye or through the skin constitutes a potential small additional factor. It is possible, however, to minimize the ingestion of dust by the simple device of exposing merely the animal's head to the dust. This has been done in certain of the studies summarized here.

From the standpoint of speed of acquiring information, moreover, inhalation experiments are time consuming. It is customary for each treatment to consist of repeated daily exposures. During treatment, furthermore, neither food nor water is available to the animals, and they are considerably restricted in action for periods of from 6 to 7 hr daily. These factors impose further arbitrary conditions on the inhalation experiments. And lastly the inhalation studies require large outlay for equipment and personnel for handling large numbers of different species of animals as already indicated.

5. ACKNOWLEDGMENTS

Grateful acknowledgment is made of the invaluable aid and continual supervision given by the following persons: Col. Stafford L. Warren, U. S. Army Medical Corps, Director of the Medical Division, a creator of projects, who furnished the impetus and saw it through; Lt. Col. Hymer L. Friedell, U. S. Army Medical Corps, Assistant to the Medical Director, a liaison between the experimental and the practical and a helpful critic of progress; Dr. Carl Voegtlin, consultant, who, through his wide experience and mature judgment, guided at each step the program of the Inhalation Section; Dr. Andrew H. Dowdy, Director of the Rochester Project, for his fair manner of handling matters of policy and his clear-sighted vision in fitting the experimental work to the practical problems; Dr. Harold C. Hodge, Chief of the Pharmacology Division, able administrator and toxicologist, who furnished the drive in getting the program under way and in sustaining its progress; Morey J. Wantman, assistant director and statistician, who, by his special training, served to shape the studies to a more fruitful and dependable outcome.

The staff of the inhalation group is especially to be commended for its countless suggestions toward the improvement of procedures and the extremely competent and cooperative manner in which the group worked as a whole throughout the entire period.

PART B. MATERIALS AND METHODS

6. EQUIPMENT: DESIGN AND OPERATION

One of the largest problems of the inhalation program was the construction of chambers for the exposure of considerable numbers of animals of several species. At the start, no exposure equipment was available from which an approximate idea of the relative inhalation toxicity of the various dusts could be gained.* Accordingly it was decided that a number of different types of chambers would be constructed for the short-term studies, which were regarded as pilot tests, and, as knowledge increased, a basis for the construction of improved types of chambers for the chronic exposures would be provided. Unfortunately, inhalation is not a sufficiently well-established toxicologic procedure to provide standardized equipment.

A survey of exposure chambers was made throughout the country, where through the courtesy of the staff of four institutions,† information was drawn on the design of existing types of chambers. The problem remained of adapting the exposure chamber and equipment for uranium dusts of high densities, which ranged from 2.4 for the nitrate to 10.5 for the dioxide. How this problem was met and how uniform distribution and diverse concentration levels of the heavy dusts were maintained are the subjects of the following section.

6.1 Design and Operation of Exposure Chambers. (Report by Sgt. Neil Murphy; work by Sgt. Murphy, Capt. G. E. Goring, *et al.*) The exposure units varied in size, in type of construction, and in provisions for the exposure of animals.

(a) Number and Size. Seven units were used for the short-term studies, three of which were later rebuilt and enlarged and others added, making a total of ten exposure units used at different periods of the studies that included the chronic experiments. Two additional smaller units of all-metal construction were contributed by the University of Pennsylvania and used for the small-scale emergency work and for special tests.

The units ranged in size from those used for the emergency work, which were rectangular in shape with a volume of 35 cu ft, or from 4-ft cubic units that could be set on tables, to approximately 9-ft cubes having a volume of 650 cu ft.

(b) Type. The design of the units was of two types, one to give complete animal exposure, the other, of more complex construction, for head exposure of rabbits, cats, and guinea pigs, and also body exposure of other animals. The method of head exposure, though little used, is not new; it has been used in Europe and in certain laboratories in this country.

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* Fortunately, by the time the exposure units were ready for operation the relative oral toxicities of certain compounds were available from experiments long since under way by Hodge and Tannenbaum.

† Appreciation is expressed for the invaluable help given by Dr. H. H. Schrenk and Mr. S. J. Pearce and associates of the U. S. Bureau of Mines, Pittsburgh, Pa.; by Dr. Don Irish and staff of the Dow Chemical Company, Midland, Mich.; by Dr. Lawrence T. Fairhall and coworkers of the National Institute of Health, Bethesda, Md.; and by Dr. Joseph Treon of the Kettering Laboratories, Cincinnati, Ohio. Drs. Schrenk and Treon and Mr. Pearce were later consultants to this section.

In general, provision was made for conditioned air to enter through one or more ducts in the roof and to be withdrawn by a Rotoclone (Type D, American Air Filter Co., Inc.) through ducts provided with filters located at the four corners near the floor level. Fans were placed at suitable sites in the unit and at a direction calculated to give greatest turbulence throughout the unit. Partitioned wire-screened cages on rollers were provided for housing the animals during exposure. Individual boxes that slid into gasketed openings on racks were provided for the head-exposed animals.

(c) Features of Construction and Operation. The chambers were of three construction types: (1) wood and glass with transite lining; these included the majority of the units and were most suitable for the introduction of inactive uranium dusts, e.g., the dioxide and also the tetrafluoride and tetrachloride, at low concentrations; (2) metal-lined wooden-framed units for the use of corrosive dusts, e.g., the hexafluoride at low concentrations; and (3) all-metal units for highly corrosive vapors or dusts, e.g., high concentrations of the hexafluoride and the tetrachloride. The chambers for the most part were of frame construction with ½-in. plywood on the walls and ceiling and ¼-in. transite on the floor and were provided with windows for light and observation of the animals. Corrosion-resistant paints were used to minimize maintenance in units operating on inert or mildly corrosive compounds and to allow greater ease in cleaning. Where appreciable corrosion was anticipated, as with UF_6, the chambers were lined with thin sheet copper or were made entirely of Monel metal. Air-inlet and exhaust lines were of galvanized sheet-metal pipe or of copper tubing, a typical installation utilizing an inlet tube 5 in. in diameter and four exhaust lines each 3 in. in diameter. Air circulation within the chambers was increased by the use of one or more propeller-type fans installed near the roof. Facilities for cleaning air exhausted from the chambers included cloth filters for removal of dust and circulating liquid scrubbers for absorption of vapors. A typical exposure unit, with a volume of 280 cu ft, utilized an air-replacement rate of 140 cfm, equivalent to 30 air changes per hour. After filtering, the fresh air supply for the units was cooled to 65°F dry-bulb temperature, which, combined with heat gains within the unit, resulted in an average chamber temperature of 75°F. Humidity was not controlled but was recorded.

(d) Costs. A budget of $10,000 was allotted for each exposure unit. This figure included the building of the chamber, equipping with dust feeds, motors, fans, flues, air-conditioning and dust-sampling equipment, and exposure cages. The construction of these items, except animal cages and air-conditioning units, was performed in shops of the project building.

(e) Full-exposure Unit. A representative full-exposure unit, with a floor measuring 7 by 6½ ft and a height of 6 ft, is shown in Fig. 10.1.

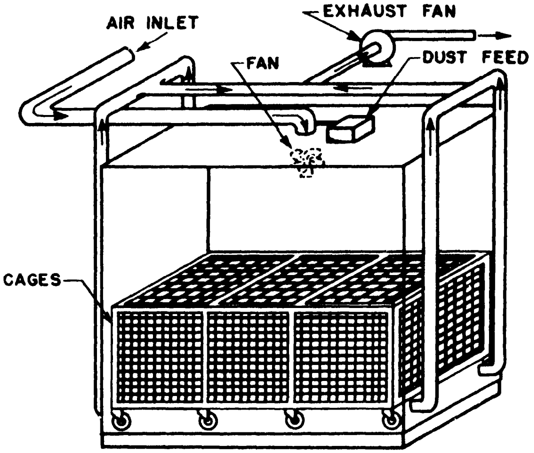

Fig. 10.1 — Exposure unit.

In this type of unit no special precautions were taken to prevent animals from ingesting dust from the fur or cages in most experiments except that the cages were washed thoroughly after each exposure period. Each of the three-cage units, shown in the above illustration, contained sections for several species of animals. For example, one of the cage units contained four dog cages of 1-in.-mesh screen, 12 rabbit or guinea pig cages of ½-in. screen, and six rat or mouse cages of ¼-in. screen. Certain of the earlier inhalation experiments were conducted in exposure units of this type; later the large cages were replaced by smaller units with individual animal compartments.

(f) Head-exposure Unit. To minimize the ingestion and the possible absorption of dust through the skin, provisions were made in a few of the units to expose only the heads of the animals. Fig. 10.2 is a view of the inside of a chamber showing, in addition to the exposure cages in the center, the heads of rabbits projecting from the side walls in the foreground and guinea pigs in the background. A photograph of the outside wall, Fig. 10.3, illustrates empty rabbit cages in the sliding

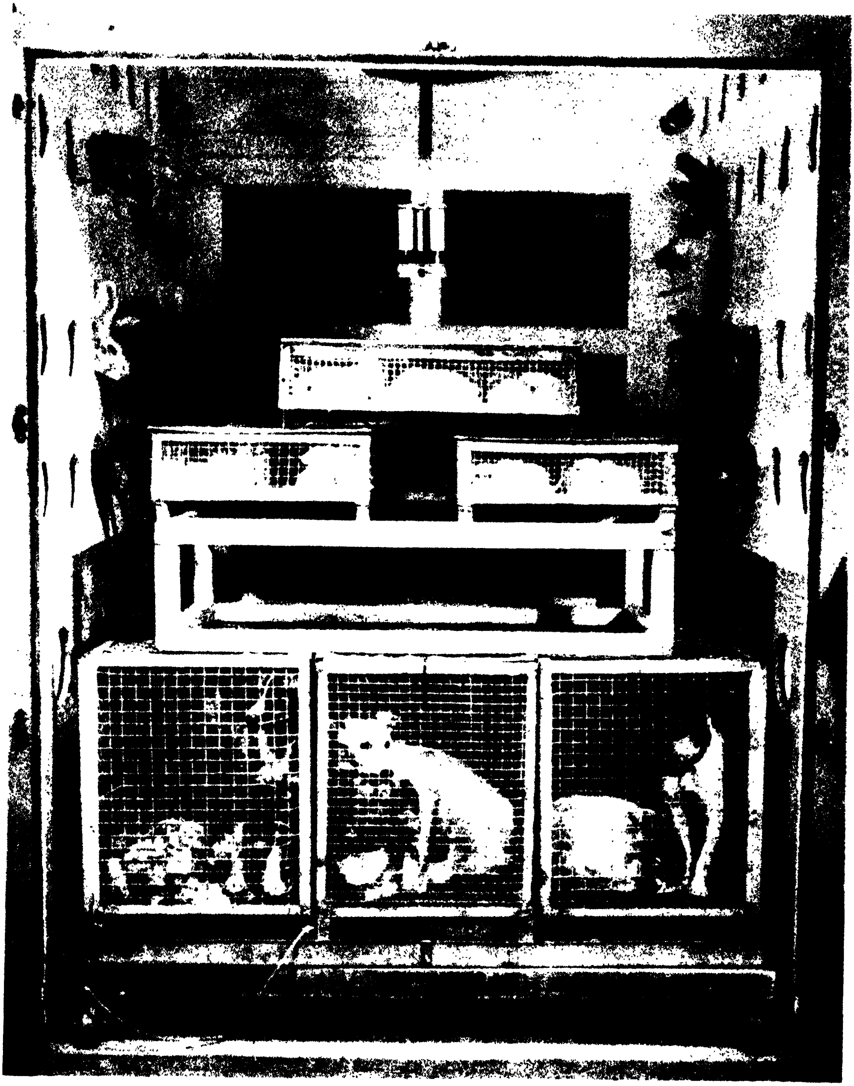

Fig. 10.2—Head-exposure unit, inside view.

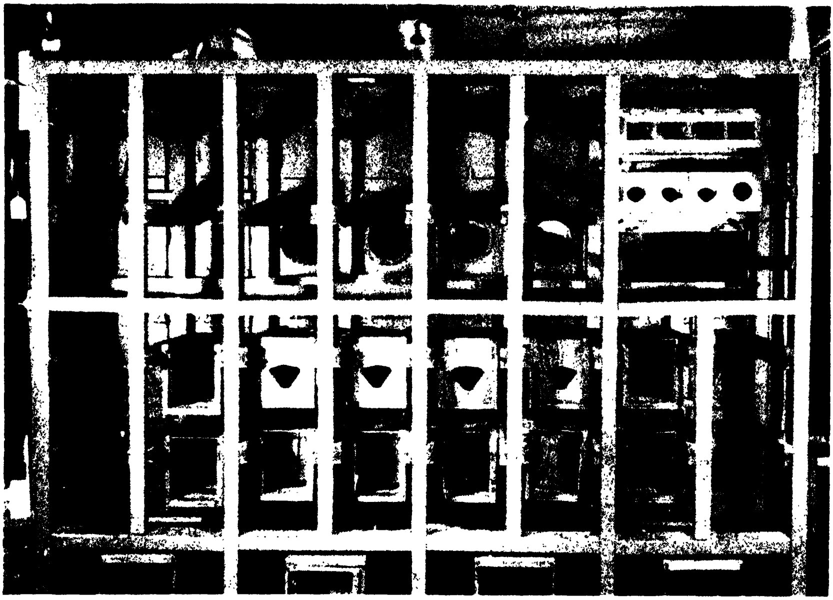

Fig. 10.3—Head-exposure unit; outside view showing open ports, cage racks, and cages for rabbits and guinea pigs.

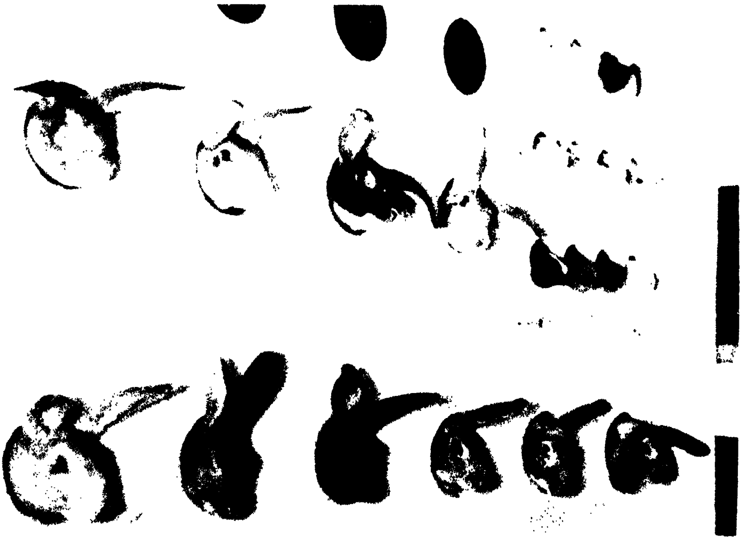

Fig. 10.4—Inside view of exposure chamber with head-exposed animals in place.

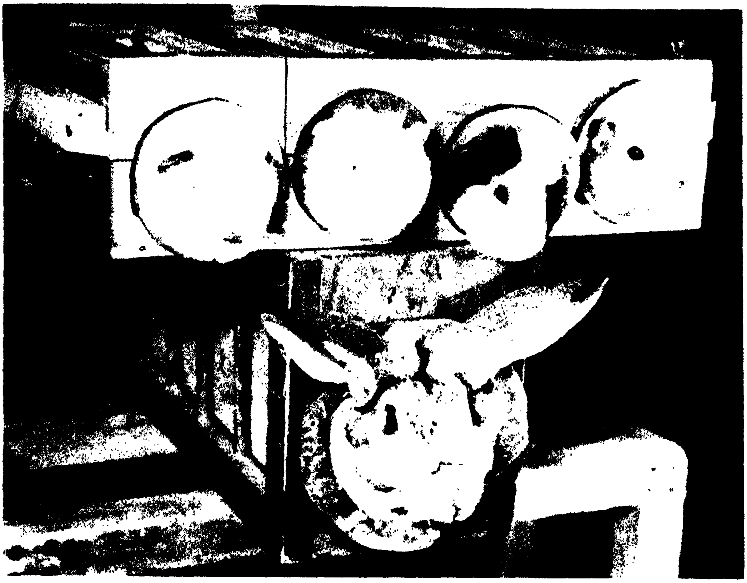

Fig. 10.5—Close-up view showing guinea pigs and rabbits in head-exposure cages with rubber neck-gaskets.

racks in the bottom tier, and, in the center of the tier above, four cages in reversed position showing the stock in which the neck was held. Also to be seen are the doughnut-shaped rubber gaskets against which the cages are pressed. Cage units for guinea pigs are located in the upper right portions of the illustration. Close-ups of the heads are seen in Figs. 10.4 and 10.5, the latter showing the pliable dental-dam rubber gasket used to minimize air leakage around the rabbits' necks.

6.2 Dust-feed Mechanisms. The design of workable dust feeds created a considerable problem because the properties of the 12 uranium dusts employed differed sufficiently so that no one model was satisfactory for all. Dust-concentration requirements for many of the inhalation studies demanded that dust be fed into the air inlet of the chamber at controlled continuous rates, often considerably less than 1 g/hr. Owing to the range of low feed rates required and the highly hygroscopic characteristics of many of the dusts, early trials with electrically vibrating devices, hopper-fed rotating gears, and the U. S. Bureau of Mines "Dust Hoist"[7] were unsuccessful. In the following paragraphs three devices that have proved to be reasonably consistent in supplying dust to the exposure units are described.

In connection with the preparation of the compounds to be used in the feed mechanisms, the dusts, with the exception of UCl_4, which was not ground, and the nitrate, which was ground once, were ground twice in a micropulverizer, resulting in an average particle mass size of the order of from 1 to 3μ.

(a) Pressure Feed. The pressure feed, Fig. 10.6, consisted of an accurately bored lucite cylinder serving as a dust reservoir and a rotating tube, the lower end of which was fixed to a brass collar. The collar, a sliding fit inside the lucite cylinder, contained several small holes, and was provided with ⅛-in. rakes on the bottom. In operation, nitrogen at a metered rate of from 0.3 to 2.5 liters/min was fed into the upper portion of the lucite cylinder and flowed downward between the rotating tube and the cylinder to pass through the 0.031-in. holes

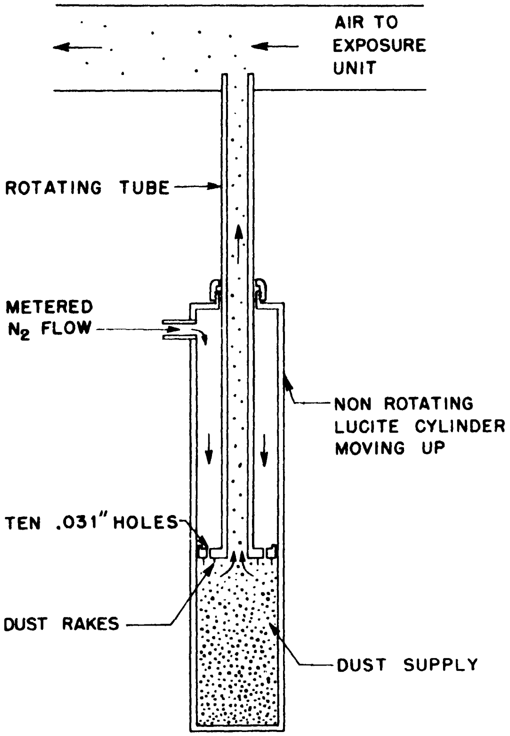

Fig. 10.6—Pressure dust feed.

into the dust reservoir. Dust in the reservoir, agitated by the incoming nitrogen and by the rakes, was carried upward inside the rotating tube into the air stream passing into the exposure unit. In order to maintain a dust supply directly below the brass collar, the lucite cylinder was geared to a variable speed Lee motor that moved the cylinder upward at a velocity that could be varied within the range of from 0.1 to 1.2 in./hr. Also geared to the motor was the inner tube with attached collar that rotated at a speed range variable between 0.3 and 4.0 rpm.

This type of feed device has been utilized in inhalation experiments of UO_2F_2, UO_2, UF_4, $Na_2U_2O_7$, and ore, achieving dust concentrations in the chamber with a standard deviation of roughly ±20 to 25 per cent. In the study utilizing $Na_2U_2O_7$, in which measurements were made of the amount of dust that reached the chamber atmosphere relative to that introduced into the air stream from the pressure feed, it was found that about one-third ultimately reached the chamber atmosphere and that about two-thirds was deposited in the duct work and chamber walls.

(b) Ball-mill Feed. Figure 10.7 is a sketch of the main features of the ball mill showing (1) the drum, which rotated at a controllable speed between 1 and 10 rpm; (2) the baffles and steel balls within the drum; and (3) the nitrogen-inlet and dust-outlet connections. As the drum rotated, the suspension of fine dust particles within the drum was carried from the drum and through the small inner tube into the air steam entering the exposure unit.

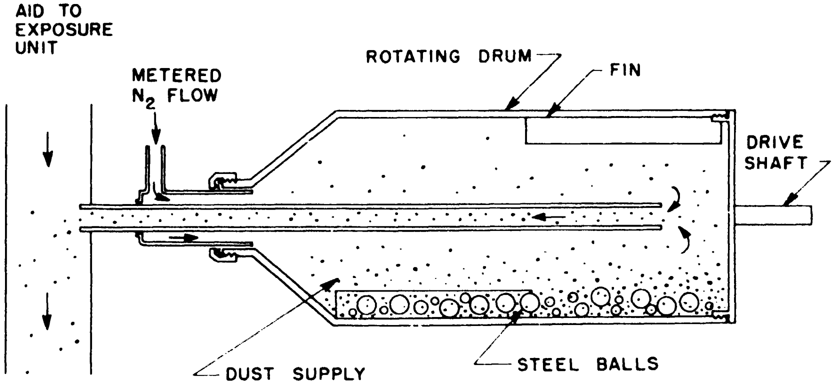

Fig. 10.7 — Ball-mill dust feed.

Designed primarily to be used with UCl_4, an extremely hygroscopic material that could not be satisfactorily ground in the micropulverizer, the ball mill served the dual purpose of a slight grinding of the dust in a dry atmosphere and feeding the suspended particles into the chamber.

(c) Agitated Elutriator. The mechanism used to feed uranyl nitrate, Fig. 10.8, was a glass flask vibrating in the vertical-plane at about 400 to 600 cycles per minute with a 1-in. stroke. The dust particles thus scattered within the flask were carried into the air inlet of the chamber by a metered stream of nitrogen. Like the pressure-feed and ball-mill mechanisms, it was usually necessary to exclude room air and moisture from the system by blocking off the inlet- and outlet-nitrogen connections when the feed was not being used. With this precaution it was possible to prepare and maintain a dry, loose dust that

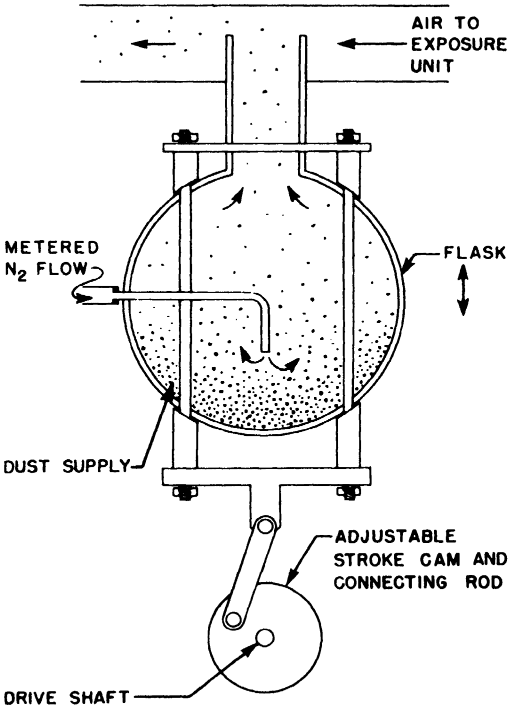

Fig. 10.8 — Agitated-elutriator dust feed.

could be aerated easily and maintain a fairly constant output. Dust concentrations within the chamber established by use of the elutriator displayed a standard deviation of about ±20 per cent at a majority of the levels.

(d) UF_6 Feed Systems. Of the uranium compounds studied, UF_6 was the only material introduced into the chamber as a vapor, the other compounds being fed in the form of a dust. Since in room air UF_6 is rapidly hydrolyzed to UO_2F_2 and HF, it was necessary to exclude all traces of moisture prior to injecting the vapor into the air inlet to the chamber. In order to minimize corrosion, tubing, valves, and cylinders were of copper or Monel, using either silver solder or 95/5 tin-antimony solder for joints and connections.

(e) UF_6 Vaporization Method. This method consisted of (1) a loading operation in which UF_6 vapor was condensed upon Monel turnings in a U tube and (2) a feeding step in which nitrogen was passed through the U tube, carrying UF_6 vapor to the exposure unit.

In the loading operation, Fig. 10.9, UF_6 vapor at 200°F and 50 psia (pounds per square inch of absolute pressure) was transferred from the supply cylinder into the evacuated vapor cylinder of known volume.

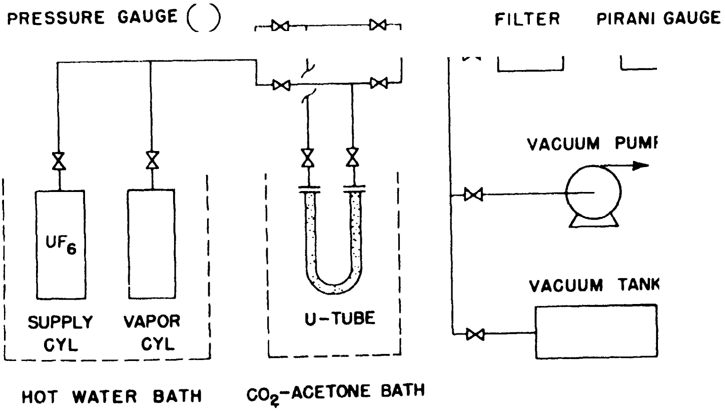

Fig. 10.9 — Uranium hexafluoride loading unit.

After closing the supply cylinder valve, the UF_6 in the vapor cylinder was allowed to condense in the U tube, the latter being maintained at −70°F in a CO_2-acetone bath. After substantially all the vapor had been condensed, the U tube was disconnected from the loading system and inserted into the feed system preparatory to revaporizing the material into the exposure chamber.

In the feeding operation, Fig. 10.10, the U tube was immersed in a cold bath at a thermostatically controlled temperature ranging from −20 to 40°F, depending on the desired UF_6 vapor pressure. The UF_6 vapor in partial equilibrium with the solid at the bath temperature was continuously removed from the tube by passing through a small equilibrium coil in the bath and then flowed through a steam-jacketed nozzle into the air inlet of the exposure unit. Although the steam-heated nozzle decreased the formation of solid hydrolysis products at the nozzle tip where the vapor was mixed with moist air, periodic cleaning of the tip was necessary.

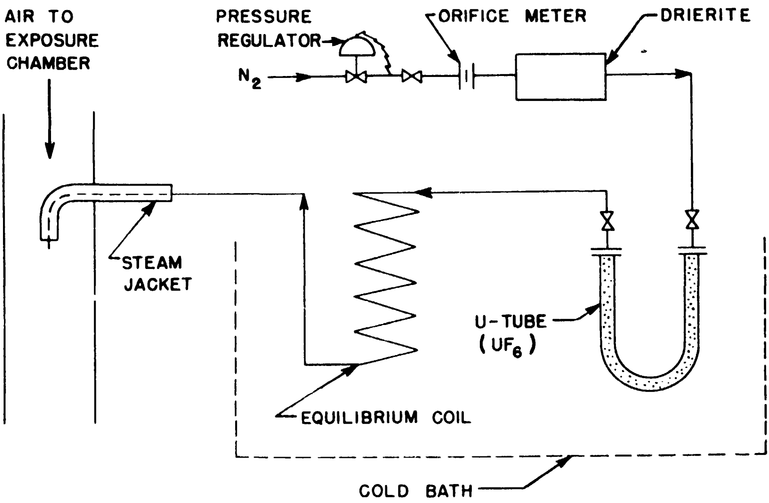

Fig. 10.10—Uranium hexafluoride feed system (schematic), vaporizing method.

(f) UF_6 Blending Method. For experiments requiring chamber concentrations less than 1 mg/cu m, it was found that the method shown in Fig. 10.11 was satisfactory, requiring less supervision and maintenance than the system described in the foregoing paragraphs. At intervals of from 7 to 14 days, depending on the rate of feed required, one or both of the blending cylinders shown in the flow plan were charged with a mixture of UF_6 vapor and nitrogen. After thorough drying of the blending cylinder and connecting tubing at a vacuum of 100 μ, UF_6 vapor at a temperature of 45°F was allowed to flow from the supply cylinder in the bath into the evacuated blending cylinder. Knowing the volume of the blending cylinder and the indicated vapor pressure of UF_6 (0.6 psia, approximately) the weight of UF_6 was calculated. The vapor was then diluted with oil-pumped nitrogen to a total pressure ranging between 50 and 200 psia, depending on the dilution required. The blend was then metered through a rotameter into the air inlet of the chamber. To minimize hydrolysis at the tip of the concentric nozzle an auxiliary stream of nitrogen was passed through the annular space around the UF_6 flow line.

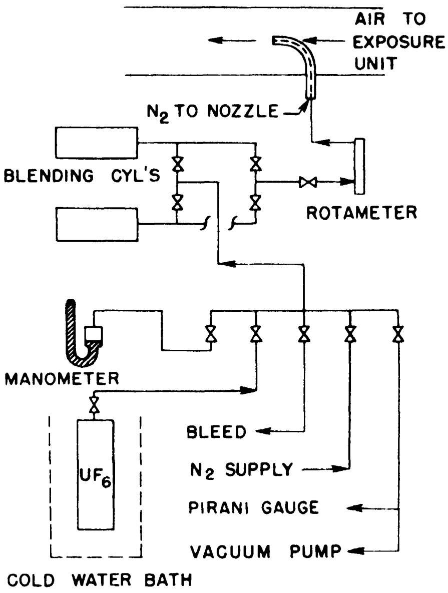

Fig. 10.11—Uranium hexafluoride feed system (schematic), blending method.

The materials used in this system as well as the equipment discussed in Sec. 1.2 included cylinders of brass or Monel, copper tubing and fittings, and Kerotest brass diaphragm valves. Cooling for low-temperature baths was obtained by the use of conventional motor-driven refrigeration compressors, and vacuum requirements were met by the use of ½-hp Kinney rotary pumps.

7. MATERIALS

7.1 Source and Number of Uranium Dusts. Eleven uranium compounds, of which most were of a high degree of purity, and one ore of high uranium content, the high-grade ore of industry, were from the same shipments used in other studies on the project (Table 1.2). The uranium materials were removed from lots during the process of manufacture, thus ensuring the identity of the material in the experimental studies with that encountered by workmen as dusts during their daily work, an important practical consideration. The following compounds of uranium were used:

Four oxides: the brown dioxide, UO_2; the yellow-orange trioxide, UO_3; the pale-yellow, fluffy, macroscopically crystalline peroxide, $UO_4.3H_2O$; the black, dense tritaoctoxide, U_3O_8.

Three fluorides: the readily hydrolyzed gaseous hexafluoride, UF_6; the green solid tetrafluoride, UF_4; the pale-yellow uranyl fluoride, UO_2F_2.

Two diuranates: sodium diuranate, $Na_2U_2O_7.1\frac{1}{2}\ H_2O$, an orange-brown solid; ammonium diuranate, $(NH_4)_2U_2O_7.4H_2O$, a deep-yellow solid.

One chloride: the green, extremely hygroscopic tetrachloride, UCl_4.

One nitrate: the yellow nitrate hexahydrate.

High-grade ore: the number and quantity of the constituents of this ore are given in Table 1.1.

7.2 Preparation of Uranium Dust. As a determinate of toxicity by inhalation, size of the dust particle has long been recognized as a factor of major importance.[14,15,16] As an upper limit, particles of a diameter greater than 5 μ have been considered physiologically insignificant because of their inability to penetrate into the pulmonary alveoli; as a lower limit 0.2 μ, because of their tendency not to be retained in the lung. The dusts on which these conclusions have been reached are insoluble nonuranium materials. Some preliminary results of the present work on the effect of particle size on the toxicity of uranium dusts are given in Sec. 8.3. The particle size of dust, moreover, is an important factor in determining the design and operating characteristics of dust feeds. And, lastly, the rate of solubility of dust is influenced in a major way by the degree of fineness of the dust.

In view of the many important factors that are influenced by particle size, much consideration was given to the preparation of dusts of extremely fine and uniform size. To this end a small factory-size model grinder, a Bantam Mikropulverizer (Pulverizing Machinery Company) was used. This machine is similar to those employed in the manufacture of fine face powders. A hand-operated screw mechanism directed the coarse uranium dust onto several electrically driven and rapidly rotating baffles of the pulverizer. The ground material passed out through a fine wire screen and was collected in a paper bag. To obtain the size considered suitable for the dust-exposure studies, the dusts were given a second grinding in the machine. As a result of this treatment all the dusts were remarkably uniform and possessed a median particle size of about 0.6 μ. An exception to this was the nitrate, which was ground only once, owing to the low melting point of the crystal hydrate (m.p. 62°C), and to the heat generated in the process of grinding. A complete discussion of the results of particle-size measurements is given in Sec. 8.2.

7.3 Characteristics of the Dusts. The purity of the dusts as determined by their uranium content after grinding, preparatory to use in the exposure studies, is shown in Table 1.2. Included also in Table 1.2 is a list of the probable impurities of major importance in the uranium compounds. Brief presentations of other characteristics of the dusts important to the inhalation studies follow.

(a) Form. Of the 12 dusts used, the peroxide was the only macroscopically crystalline material. This material after grinding possessed a long axis approximately three times that of the short axis. The nitrate was distinguished from other dusts by the fact that before its use in the dust feed it required desiccation to constant weight in which process it lost 2 molecules of water of crystallization, reducing it to the form $UO_2(NO_3)_2.4H_2O$. In the course of its circulation in the exposure chamber the dust regained water, at the same time acquiring the tendency to aggregate and form large crystal droplets. Uranyl fluoride used in the anhydrous form behaved similarly in the exposure unit, also acquiring water but showing less tendency to aggregate than did the nitrate. Uranium hexafluoride, a solid under pressure with a vapor pressure of 210 mm at 35°C, instantly hydrolyzed in the presence of moisture in the chamber, producing in stoichiometric amounts an extremely fine fume of uranyl fluoride and gaseous hydrogen fluoride according to the equation

$$UF_6 + 2H_2O \rightarrow UO_2F_2 + 4HF$$

The tetrachloride, though crystalline in the anhydrous state in which it was introduced into the exposure unit, behaves similarly to the hexafluoride in that it hydrolyzes with considerable rapidity in the moist atmosphere of the exposure unit forming the following hydrolysis products to a greater or lesser degree: UO_2Cl_2, $UOCl_2$, UO_2, HCl. As far as has yet been ascertained, no change occurred in the remainder of the uranium dusts during their distribution and circulation in the exposure chambers prior to inhalation. The form of these substances appeared to be a mixture of microscopic and broken crystals.

(b) Density. The extremely high density that characterizes a large number of the uranium compounds contributed substantially to the problems involved in introducing the dusts into the exposure chambers and maintaining their uniform concentration and distribution. Of the dusts employed, uranium dioxide possessed the highest density, 10.5; that with the lowest density was the uranyl nitrate hexahydrate, 2.75. The other materials possessed intermediate density.

(c) Particle Size. As a result of the grinding, the median particle-mass size was comparatively uniform for all dusts except the nitrate, which, for the reasons mentioned above, tended to display larger particle sizes. It should be emphasized here that these sizes were determined on dusts circulating in the exposure units in the presence of the experimental animals. Size-mass distributions are shown in Table 10.2 (in Sec. 8.2) in a separate section devoted to particle-size measurement. The median mass-size values of the majority of the dusts was from 1 to 3 μ.

(d) Purity. Eight, and possibly nine, of the eleven chemically prepared uranium compounds were pure, i.e., the analytically obtained values (chemical determinations made by the staff of the Inhalation Section) for uranium content agreed with that calculated from theory within 0.5 per cent. The salts of highest purity were the dioxide, tritaoctoxide, hexafluoride, and uranyl fluoride. The close agreement of the analyzed value with the theoretical in the case of the peroxide and the sodium and ammonium diuranates may be more apparent than real, the formula for the crystal hydrate of these materials having been determined on the assumption that the disagreement between that calculated for the anhydrous form and the hydrated form given in Table 1.2 arose from the number of moles of water shown. The formula for the hydrate of the peroxide is commonly given as $UO_4.2H_2O$ instead of the 3 moles given in the table. The diuranates occur with various amounts of water and in addition frequently occur as a mixture of polymers of the type formula $(Na_2O)_x2(UO_3)_y$ depending upon the method of preparation. The low uranium content of the tetrachloride is questionable because of the extreme difficulty of obtaining an absolutely anhydrous sample of this salt for analysis. The purity of different lots of the nitrate varied widely, arising chiefly from the varying amounts of free nitric acid. Excess acid was removed in vacuo before grinding. The analysis given for this salt in Table 1.2 is for the tetrahydrate, the stable form resulting from the desiccation to constant weight in vacuo. The tetrafluoride and the trioxide were the most impure of the compounds used as judged by the uranium analysis. The former showed a content of uranium greater than the theoretical, which was undoubtedly contributed by the dioxide impurity. The tetrafluoride, however, was shown by polarographic analysis and toxicity tests to contain from 2 to 3 per cent of the highly toxic uranyl fluoride. The trioxide undoubtedly contained sufficient amounts of the peroxide to lower its uranium content; the latter compound, however, was of the same order of toxicity as the trioxide and consequently created no problem in the interpretation of toxicologic response. Cylinders of the hexafluoride were claimed to contain no more than 3 per cent hydrogen fluoride, but this impurity was reduced to a negligible amount in the process of in vacuo transference preparatory to its use in the exposure studies.

High-grade ore presented a special case in regard to composition. This ore contained approximately 60 per cent uranium by analysis and, in addition to 47 per cent U_3O_8 and 21 per cent UO_2, contained 16 other constituents each in amount greater than 0.2 per cent, of which lead oxide was 6 per cent. Next to the afore-mentioned two uranium oxides, the lead oxide was probably the most important toxicologic factor of the ore to be reckoned with. A number of other constituents were present, each in amount less than 0.2 per cent. These as well as the 18 major constituents of the ore are listed in Table 1.1.

(e) Solubility. The solubility rate of 10 of the uranium compounds was determined in beef serum at 37°C, according to the following procedure. The compound, ground to the uniform particle size described above, was placed in 10 ml of serum to the extent of 10 mg of uranium; the mixture was placed on a shaker and rotated. Samples of supernatant fluid were removed for polarographic analysis after ½, 1, 2, 4, 12, and 24 hr. The results of the average solubility rates are shown in Fig. 15.1.

The four oxides, the two diuranates, and the tetrafluoride showed the slowest rate of solubility of all the uranium compounds in serum. Highest rates of solubility were shown by the uranyl nitrate and fluoride. The uranium tetrachloride and peroxide gave intermediate values.

8. METHODS AND PROCEDURES

This section describes the procedures involved in the animal-exposure studies. These are grouped under two chief headings: (1) the mechanical, physical, and chemical controls used for regulating the preparation and maintenance of the dusty atmospheres; (2) the toxicological procedures, including the preparation of the animal material, used in the design of the experiments. Because these procedures involve numerous separate details, the subjects under each heading will be presented in the sequence in which they were performed during the experiments. In establishing the proper conditions for the experiments, both the exposure-chamber tests and the preparation of the animals proceeded simultaneously. For the purposes of presentation, however, the methods of developing and maintaining a dusty atmosphere of uniform concentration and particle size will be discussed first. In certain instances where the presentation will be aided, the results of some of the tests made later during exposure studies will be given.

8.1 Establishment of Dust Concentration. The maintenance of a uniform concentration of uranium dust within the exposure chamber depends on the rate of introduction of the dust from the feed into the chamber, the density and range of particle size of the dust, the air turnover and circulation within the chamber, and the humidity of the atmosphere. All variables were controlled in so far as possible. As already indicated, the characteristics of the dust and the rate of air turnover in the chamber were relatively easily controlled, and in most instances the rate of introduction of the dust from a particular type of feed was comparatively uniform. The humidity, changing with atmospheric conditions and unregulated in the exposure unit, was the most variable factor. Its greatest effect was felt with dusts that were hygroscopic, which include the nitrate, tetrachloride, and uranyl fluoride. The humidity, although variable, was not the major factor in governing the dust concentration within the chamber. More important was the rate of introduction of the dust and the air turnover and circulation within the chamber.

The effect of these variables on the dust concentration was established by repeated tests over a period of days during the time that the animals were being prepared for exposure. It was not uncommon that a period of weeks was required before the results of the tests were considered satisfactory.

(a) Method of Sampling. Concentration of the uranium dust within the chamber in most of the studies was determined by means of sampling with a filter-paper dust sampler, at a constant air flow, a known amount of chamber air containing the dust. This type of sampler was modeled after that described by Fairhall.[13] The method of its use, however, differed in the present work; the sampler was attached to the end of a long rod that protruded into the chamber, allowing samples to be taken at any location rather than at the wall of the exposure unit. A similar sampling unit also used in inhalation work is shown schematically in Fig. 10.12.

The amount of air sampled in the course of these studies was from 0.5 to 3 cu m at an air-flow rate of 1.5 cfm (0.0425 cu m). For dust concentrations approximating 0.05 mg/cu m, 3 cu m of air were sampled,* requiring a sampling time of about 90 min; for concentrations of from 10 to 20 mg/cu m, a 15-min sample was taken. Intermediate concentrations required corresponding lengths of time. The method of sampling consisted merely of impinging the dust on a circle of Whatman No. 41 filter paper. The difference in the weight of the filter paper before and after sampling furnishes an estimate of the amount of dust in the sampled air volume, provided the quantity of nuisance dust in the atmosphere is low.†

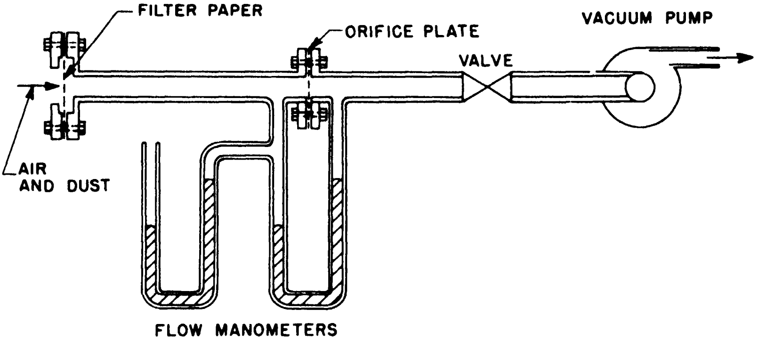

Fig. 10.12 — Filter-paper dust-sampling unit.

(1) Chemical Analyses of Dust Sample. Accurate estimation of the amount of dust on the filter paper was obtained by quantitative chemical analysis of the sample by a modification of the ferrocyanide acetic acid method of Bénard and Tessier (Chap. 2). The time required for the colorimetric analysis of each dust sample is 45 min. The more approximate gravimetric analysis allowed a more rapid control

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* In some of the early studies sampling was performed by means of either an electrostatic precipitator or a Greenburg-Smith impinger or both.

† This factor was reduced to approximately 0.02 mg/cu m of air by the use of oil-sprayed precipitron plates in the air-conditioning system of the chamber. This represents approximately the minimum obtainable owing to the contribution from animal activity during an exposure.

of the chamber-dust concentration and was found by statistical analysis* to be sufficiently accurate to obviate the need of more than an occasional chemical analysis of those dusts unaffected by moisture of the air, e.g., the dioxide, trioxide, tetrafluoride, and ore.

During the actual operation of the units, sampling for concentration was performed at periodic intervals, usually hourly, from the start to the finish of the 6-hr exposures. The first sample was begun within 15 min after the introduction of the dust into the unit.† A final daily sample was taken just prior to stopping the dust feed. Samples were taken uniformly at one well-chosen location in the unit. Thus the mean dust concentration for a day's exposure was based on from four to seven separate determinations. The mean was calculated by statistically weighting each concentration by the interval between samples. From these data the frequency distribution of a dust sample for a given exposure day was computed.

(2) Chamber-dust Concentration. For each level studied the dust concentration was expressed as an over-all mean, and the variation from the mean was expressed as a standard deviation. These values were obtained by averaging the daily mean values that had been weighted for the time between which the samples were taken. The values of the mean concentration and standard deviation are presented for each dust level studied. Use of the standard deviation as a method of describing the concentration is often misleading because the distribution is usually skewed whereas the standard deviation assumes a normal distribution. Accordingly, the method of plotting the frequency distribution curves of the dust samples was also employed. Such curves show the relative frequency with which dust concentrations of a given class interval were observed throughout the period of exposure.

In this section are presented frequency-distribution diagrams of representative dust-exposure concentrations illustrative of the four types of dust feeds used in exposure studies: (1) ball-mill, (2) vapor-dilution, (3) pressure, and (4) agitated-elutriator.

1. Figure 10.13, upper left, shows the frequency distribution of the concentration of the ball-mill dust-feed samples of UCl_4 and its decomposition products for two levels, 0.05 and 0.2 mg U/cu m plotted

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*The correlation of 76 samples analyzed by both methods was .94. On the average the gravimetric method gave results that were 8.3 per cent higher than the colorimetric method, the higher value by the former method undoubtedly being attributable to the presence of nuisance dust and moisture.

†It was found that the dust concentration attained the desired level within from 5 to 10 min after start of the dust feed.

from 588 and 833 individual samples, respectively, taken during a 7-month period. The curves illustrate the functioning efficiency of the ball-mill feed used to introduce and maintain the concentration of this and certain other dusts used in the studies. It is seen that at the lower level 86 per cent of the samples were within ±60 per cent of the desired level of 0.05 mg/cu m; at the higher level 78.4 per cent were within ±50 per cent of the desired level of 0.2 mg/cu m. The graphs

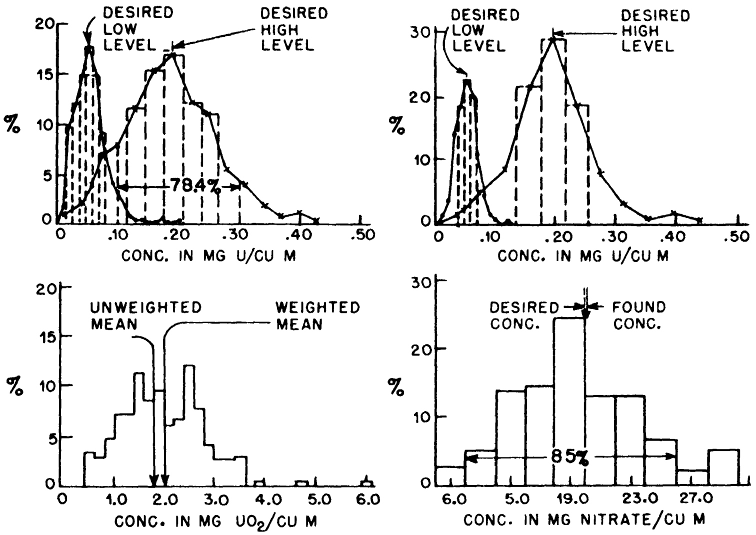

Fig. 10.13—Representative frequency-distribution curves of dust concentration. Upper left, uranium tetrachloride atmospheres (two levels) by ball-mill feed; upper right, uranium hexafluoride atmospheres (two levels) by blending method; lower left, uranium dioxide atmosphere (one level) by pressure feed; lower right, uranyl nitrate atmosphere (one level) by agitated-elutriator feed.

further show that, although the frequency curve of both levels overlapped, the number of the samples of the low level greater than 0.1 mg/cu m was only 3.6 per cent and represented a negligible number near the desired level of the higher concentration, a factor of extreme importance in evaluating the pathological response in the animals. Thus the toxic response between two such closely lying levels of dust should be capable of differentiation in so far as the definition of the levels is concerned.

2. An example of an excellent distribution of the concentration of dust produced by vapor dilution is given in Fig. 10.13, upper right, wherein are shown frequency-distribution results of the fume UO_2F_2 derived from gaseous UF_6 at two levels of 0.05 and 0.2 mg/cu m for a 5-month period. It is seen that both curves peak sharply at exactly the desired levels, with an extremely narrow spread, and with little overlapping between the two levels. These curves illustrate the greater uniformity with which concentrations of dust derived from the introduction of gaseous material are developed and maintained.

3. Illustrative of results obtained with the pressure feed using UO_2 dust at a level of 2 mg/cu m for a 20-day period are those shown in Fig. 10.13, lower left. Somewhat less satisfactory results from this feed were obtained even at ten times higher concentration than were obtained with the ball-mill feed. Although the weighted-mean concentration was within 0.2 mg/cu m of the desired concentration, two peaks were observed at either side of this level, and occasional samples were as high as 6 mg/cu m. However, 77 per cent of the samples were within ±50 per cent of the desired 2 mg/cu m.

4. A uniform distribution of dust concentration was obtained with the agitated elutriator feed employing the nitrate dust at 20 mg/cu m. The weighted-mean concentration was 20.58 mg/cu m, approximately that desired. The standard deviation was 4.24 mg/cu m (Fig. 10.13, lower right). The bulk of the samples taken were distributed evenly about the desired mean and 85 per cent of the samples were within ±30 per cent of the desired 20-mg level. This represents a somewhat better performance of this feed than was obtained with the nitrate dust at concentrations below the 20-mg level.

(3) Spot Test for Determination of Dust Concentration. In certain instances, notably the exposure studies using low concentrations of the nitrate in which the gravimetric procedure was not applicable and the chemical analysis too time consuming, or when more frequent monitoring of the concentration was demanded, a spot-test method utilizing the Aluminon* reagent[17] at pH 4.5 had a decided advantage in a rapid control of the dust concentration. Comparisons with standards were made within 1 min after the sample was taken, requiring no more than 5 min total time. Accurate control of chamber-dust concentration was thus permitted.

* Ammonium salt of aurin tricarboxylic acid, the most sensitive reagent found by us for a spot test for the uranyl ion when the reaction is carried out on filter paper. A stable and readily perceptible pink color develops with 5 μg of uranyl ion. The reaction is not specific for this ion.

(b) Test of Dust Distribution. For a given dust, assuming the rate of introduction of the material and the air turnover to be constant, the circulation within the unit becomes the next factor of importance. For this reason, test of the air circulation was made by taking air-velocity readings in the units by means of a Velometer (Illinois Testing Laboratories, Inc., Chicago) with the animal-exposure cages in position in order to locate inequalities and to ensure the absence of dead-air space. Irregularities in the circulation were corrected by judicious placement and direction of fans. Subsequent to these tests, simultaneous samples of the atmosphere were taken throughout the unit, including the cage space, one of the sampling positions remaining fixed as a point of reference. Two typical examples of such a dust-distribution study follow, one representative of a good distribution and one of a poor distribution.

A distribution study to determine the degree of uniformity of dust concentration was made in the 9-ft-cube exposure unit employing nitrate dust at 2 mg/cu m just prior to the start of chronic exposure. All animal cages were in the positions that were ultimately used in the study. Another study was done 6 months later when the animals were occupying these cages in the process of being exposed. Samples were taken at six critical positions in the chamber. Each position was sampled simultaneously with that of the reference site, a position chosen as representative of the concentration of the entire unit. A height approximately midway between the floor and the ceiling of the chamber was selected for the reference site. Four or five individual values were averaged to obtain the concentration at any point relative to that of the reference site. The results showed that for this unit 77 to 115 per cent of the nitrate dust found at the reference site was present in all parts of the unit close to the cages and indicated a good distribution of the dust. This unit was the largest of those employed on the project having the lowest animal-volume to chamber-volume ratio of any of the units and contained in addition four fans directed toward the central point in the unit. The results of similar studies made in other units are given in Table 10.1, along with data on the ratio of animal volume to chamber volume.

The table shows that the uranium-dust distribution in four of the six chambers was reasonably satisfactory, the average concentration at any point being from 79 to 100 per cent of that at the reference site. In two chambers less satisfactory distribution was obtained; at some sites in the units approximately one-quarter of the measured dust concentration was found. There was, however, some daily variation in the distribution at any point, and compensation for inequalities was made by a scheduled rotation of the animals.

The ratio of the space occupied by the animals to the interior volume of the exposure unit was well below 5 per cent for six of the seven. With such a low volume ratio and such a high air turnover (see next paragraph) within the unit, the contribution of the animals through respiratory exchange to the composition of the circulating air was negligible.

Table 10.1 — Data on Animal-volume to Chamber-volume Ratio and Dust Distribution in Exposure Units (First 30 Days of Long-term Studies)

Concentration level of compound, mg/cu m	Compound	Vol. of unit, liters	No. of animals exposed				Vol.* of animals, liters	Ratio, animal vol. to chamber vol., %	Dust distribution†	
			Dog	Rat	Rabbit	Guinea pig			Range, %	Mean, %
0.0		8,609	12	128	30‡	30‡	235	2.7		
2.0	$UO_2(NO_3)_2.6H_2O$	20,600	18	147			200	1.0	77–115	96
0.9	$UO_2(NO_3)_2.6H_2O$	7,100	19	150			190	2.7	23–79	44
0.2	UF_6	10,325	19	120	12		260	2.5	66–91	79
0.2	UCl_4	9,577	19	118	20	30	336	3.5	27–100	57
3	UF_4§	1,900	6	180	45**	36**	141	7.4	85–115	100
10	UO_2	11,800	18	150	18	30	316	2.7	67–94	81

*Average weight of animals in each chamber divided by density of 0.9; 10 per cent of body weight was used for calculating volume of head-exposed animals.

†Expressed as percentage of dust relative to that at usual sampling site.

‡15 animals were totally exposed, and 15 were head exposed.

§Also 180 mice and 6 head-exposed cats.

**All were head-exposed animals.

(c) *Air Turnover, Temperature, and Humidity*. Air flow through the chambers allowed one complete air change each 2 or 1½ min. Air-conditioned room air (containing approximately 0.02 mg/cu m of nuisance dust) was introduced in certain of the units.* The air was conditioned so that the chamber temperature was maintained at approximately 72°F throughout the year. The humidity varied from 35 per cent in the winter months to an average of 60 per cent in the summer months. During any one month there was no considerable variation in these factors.

8.2 *Measurement of Particle Size*. (By Sidney Laskin; work done by S. Laskin, A. Fiorica, M. Reid, N. Glover, and C. Horton; general consultant, A. Rothstein; engineering design consultant, C. W. LaBelle.)

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* In units in which the air was not filtered for dust but refrigerated only (Units 7, 10, and 12) the nuisance-dust concentration ranged from 0.3 to 2 mg/cu m, with an average of 0.6 mg/cu m.

(a) The General Problem. Particulate matter dispersed in the atmosphere possesses a size between that of collodial and macroscopic particles. This group, classified by Gibbs[18] as aerosols, corresponds to the accepted classification of dispersed systems in water known as "hydrosols." Aerosols include the familiar atmospheric suspensions known as dusts, fumes, smokes, mists, clouds, and fogs. Since the term "aerosol" has been frequently used to denote fogs and mists in particular, atmospheric suspensions are often classified as dusts, fumes, smokes, and as aerosols. The distinction between these groups is made on the basis of the source of the material, physical properties, and the size ranges found. All or any of these types of atmospheric suspensions are commonly produced in industrial processes. In the uranium industry, however, dusts and fumes are chiefly encountered, and therefore the discussion is limited to these groups.

Dust particles are formed as a result of a reduction in size of materials by grinding, crushing, blasting, and drilling. The composition of the particles is the same as that of the parent substance, and the sizes produced range from the visible to the submicroscopic. Fumes are formed as a result of processes involving combustion, sublimation, and condensation of inorganic material. They generally have a particle size less than 1 μ. Fumes, however, are distinguished from smokes in that the latter are of smaller size and are generally of organic origin.

(1) Dusts and Fumes in Relation to Inhalation Toxicity. Particle sizes of material dispersed as dusts and fumes may be larger than those of colloids but still are sufficiently small to exhibit many of their properties, notably a reactivity greater than that possessed by larger masses of the original substance. Comprehensive discussions on the subject are those of Drinker and Hatch[20] and Dallavalle.[21] Despite the extensive literature on the subject, the laws governing the general behavior of small particles are not fully understood. This situation exists because of the lack of basic information on elementary factors such as particle shape, particle surface, and particle size. Some general inferences, however, may be made in regard to the uranium-toxicity problem.

The division of a solid into small particles and their subsequent dispersion in the atmosphere result in two important changes. Tremendous increases occur both in the surface area of the material and in the total space occupied. Thus Drinker and Hatch[20] show that, if 1 cu cm of quartz is crushed into particles, 1 cu μ in size, there will be 10^{12} particles with a total surface area of 6 sq m as compared with that of the original block. Assuming a dust concentration of 100 million particles per cubic foot of air, 1 cu cm of material will be dispersed in an air volume of 10,000 cu ft. The effect of these changes is to intensify the chemical and physical activity of the material. These properties show in increased rates of reactions involving oxidation, solubility, evaporation, adsorption, and electrical activity. Changes in these properties may also cause subsequent changes in other physical properties. Thus the adsorption of a gas film on a particle surface may interfere with its chemical reactions and also with physical phenomena, such as wetting.

The physiological effect of air-borne particles is also intimately associated with their physical and chemical properties. Since these properties are related to particle size, the significance of particle size to the inhalation-toxicity problem is considerable. In general, small particles may be expected to be more active than large ones. Moreover the sizes effective in producing toxicity depend upon the amount of a given size retained by the animal.

Air-borne material enters the body through the upper respiratory tract, a part of the function of which is that of serving as an air-conditioning apparatus. Air is delivered to the alveoli at a modified temperature and humidity, and, under normal conditions, free from harmful concentrations of particulate matter. That the air-cleaning efficiency is not complete has been indicated by numerous toxicologic studies (see Bibliography, references 22 to 25). Particles of the order of 5 μ or larger have been reported to be completely removed by the upper respiratory tract. Some of the finer dust is not filtered and is deposited in the alveoli. The remainder is returned to the outside atmosphere. The finer particles, which are retained in the alveoli, are generally considered to be those largely responsible for producing toxicologic effects, whereas the larger particles removed in the upper respiratory tract have been considered unimportant because mechanisms exist for their continuous removal. However, evidence to the contrary exists in the case of lead.[26]

Although only limited information is available concerning the retention of dust in various parts of the respiratory tract, some data are available concerning the over-all retention within the animal. Thus retention is stated to be of the order of 20 per cent for particles less than 0.3 μ and up to 100 per cent for particles as large as 5.0 μ.[27] Retention is a function not only of particle size, shape, density and concentration, but also of the breathing characteristics of the individual. These factors show their influence in the marked variations that occur in inhalation-toxicity studies.

A striking example of the specific effect of particle size in producing toxicity is demonstrated by the finding that only freshly formed and nonflocculated zinc oxide produces metal-fume fever, whereas resuspension of the previously formed oxide results in a cloud of flocculated larger particles that cannot penetrate the alveoli and cause damage.[24] Moreover, in Sec. 8.3 it is shown that the particle size of uranium dioxide dust placed in the lungs of rats has a very marked effect on the resulting toxicity. The larger sizes (greater than 1 μ) are relatively nontoxic, whereas the smaller sizes (less than 1 μ) result in increased toxicity.

(2) Definitions and Treatment of Data. Before discussing the particle-size data, the general methods of characterizing particle size will be briefly given. Most finely particulate matter possesses irregular particle shapes for which no definite geometric diameter can be defined. Resort is made to an arbitrary diameter, rigidly defined and easily duplicated, which is the distance between the two extreme points on a particle when measured in a horizontal direction. This may represent the major or minor axis of the particle or some position between the two. This dimension, termed the "statistical diameter," has been shown to give a representative picture of the size distribution of an entire sample,[28] provided that the particles exhibit a chance distribution with respect to size and shape. However, in order to obtain a statistically valid sample of the sizes present, every particle in the field of view must be measured.

All the particles encountered in the uranium-dust atmospheres exhibit a very wide range in sizes requiring the use of a distribution curve that shows the number of particles in each size group as a function of the diameter representing that group. These are known as the "size-frequency" or "size-count" distribution curves. These curves have been shown to follow the probability law and can be defined numerically in terms of the parameters of a normal probability curve.[21,29] The equation for a normal curve applies only to distributions that are symmetrical about a vertical axis, and, since the particle-size distributions are generally asymmetrical or skewed with respect to this axis, normalization of the curve is first required. This is accomplished by expressing the sizes as logarithms.

The probability equation for the asymmetrical size-frequency distribution can be derived as

$$F(d) = \frac{\Sigma n}{\log \sigma_g \sqrt{2\pi}} \times \left[-\frac{(\log d - \log d_g)^2}{2 \log^2 \sigma_g} \right] .$$

where F(d) is defined as the frequency of observations of the diameter d. Σn is the total number of observations; d_g and σ_g are the constants that completely define the frequency distribution. The quantity d_g is called the "geometric mean" and is obtained from the following relationship:

$$\log d_g = \frac{\Sigma(n \log d)}{\Sigma n}$$

The geometric standard deviation σ_g is obtained from the equation

$$\log \sigma_g = \sqrt{\frac{\Sigma n(\log d - \log d_g)^2}{\Sigma n}}$$

The standard deviation (σ_g) of a size distribution is a measure of the dispersion of particle sizes about the mean size. For a normal distribution approximately 65 per cent of the sizes fall between the mean diameter $\pm 1\sigma$ and approximately 95 per cent of the sizes fall between the mean diameter $\pm 2\sigma$.

Because the calculation of the above parameters is rather lengthy and tedious, it is more practical to obtain them graphically by use of a log-probability grid. This grid consists of a system of coordinates based on a scale derived from the probability integral.[30] This scale is the ordinate of a graph on which the frequency is plotted, expressed as a cumulative percentage less than the stated size. The abscissa is a logarithmic scale on which the diameters of the measured particles are plotted. The theoretical probability function thus represented is a straight line, and, although under practical conditions some divergence is found at the extremes, this curve adequately represents the measured distribution.

Both the desired parameters (mean and standard deviation) may be easily and directly obtained from the curve as follows: The geometric mean is the 50 per cent or median intercept on the resultant curve, since by definition the mean bisects the symmetrical distribution curve. The geometric standard deviation is obtained from the relationship

$$\sigma_g = \frac{84.13\,\%\,\text{size}}{50\,\%\,\text{size}} = \frac{50\,\%\,\text{size}}{15.87\,\%\,\text{size}}$$

which is derived from the probability integral.

Since most toxicologic studies are made in terms of concentrations required to produce specific effects, the information on particle sizes given in terms of size frequency has little direct meaning. It is therefore necessary to express the size distribution in the more useful form in which mass frequency instead of size frequency is given for each size class. The mass frequency may be calculated from the measured sizes on the basis that the mass of a particle is a cube function of its measured diameter. The size frequency obtained for each class is weighted in terms of the cube of the average-size value representing that class, and a plot of the resulting values against size shows a distribution similar to that obtained for the size-count distribution. This distribution is known as the "size-mass distribution," the parameters of which can be obtained by the identical procedure used for the size-count analysis.

In recording size-distribution results for uranium dusts and fumes, the parameters of both types of distributions are given as the median value, M_g, and the geometric standard deviation, σ_g. The use of median instead of geometric mean is a convention adopted for convenience.

(3) Methods of Sampling and Measuring. Measurement of particle size commonly involves two procedures, sampling (the separation of dust from the air) and the measurement of particles. Some methods, however, make measurements directly on dusts suspended in the air and thus require no sampling procedure, whereas others serve simultaneously for sampling and measurement. Because standard references[20,31] are available concerning methods of sampling and measurement of dust particles, in the present discussion only the general principles involved will be considered.

At the start of the research program on uranium, certain standard sampling instruments were available that had been in general use in the industrial field. These included the electrostatic precipitator, settling chamber, thermal precipitator, Bausch & Lomb dust counter, Greenburg-Smith wet impinger, and dry impinger. Measurements were made with the optical microscope by projection onto a screen with calibrated squares as suggested by the U. S. Bureau of Mines.[31] Representative data are given in Table 10.2 and in the appropriate sections on short-term toxicity studies.

As the studies of particle size progressed it soon became evident that the methods used were subject to serious errors and that the results obtained were only approximate. The sampling methods were generally selective for only a portion of the total size distributions encountered, and they were incapable of handling some of the highly hygroscopic materials. The measuring method was inadequate because of problems of visibility and resolution.* Since the refractive index of the uranium compounds approximated that of the standard mounting media, visibility could be obtained only with an air mount as shown by Foster and Schrenk.[34] However, with an air mount the limit of resolution of an ideal microscope is only of the order of 0.5 μ, and large errors are involved in measurements below 1.0 μ. Consequently the large proportion of the size distribution of uranium dusts

Table 10.2—Median Particle Size and Standard Deviation of Uranium Dusts*

Compound	Sample No.	Concentration level of dust, mg/cu m	Median, M_g, μ	Geometric standard deviation, σ_g	Sampling method
$Na_2U_2O_7$	1	20.3	1.15	3.65	Th. prec.†
	2		0.80	2.74	Th. prec.†
	3		0.66	2.12	Th. prec.†
	4		0.59	0.57	Th. prec.†
	5		0.66	1.82	B & L‡
High-grade ore	1	36	0.74	1.89	B & L‡
	2		0.56	1.78	B & L‡
	3		0.35	0.50	B & L‡
	4		0.60	2.18	B & L‡
	5		0.54	2.21	B & L‡
$UO_2(NO_3)_2 \cdot 6H_2O$	1	2.0	0.94	1.81	Settling
	2		2.55	4.65	Settling
	3		0.50	2.40	Settling
	4		0.42	2.41	Settling
	5		0.80	2.40	Settling
$UO_2(NO_3)_2 \cdot 6H_2O$	1	0.5	0.20	10.00	Settling
	2		0.94	2.87	Settling
UO_2F_2 from hydrolysis of UF_6	1	19.8	0.41	2.19	Dry imp.§
	2		0.44	2.07	Dry imp.§
UO_2F_2 (ground)	1	0.6	0.70	2.00	Dry imp.§
UO_4	1	19.5	0.86	1.69	B & L

*Measurements made by means of a projection microscope.

†Thermal precipitator.

‡Bausch & Lomb dust counter.

§Dry impinger.

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* General discussions of visibility and resolution can be found in any of the standard books on the microscope such as Chamot and Mason's [32] and Shillaber's.[33]

below this limit was never measured, although it was known to be of marked toxicologic significance.

It was evident that major improvements in the methods of measurement were required if size data useful in evaluating toxicologic effects were to be obtained. Desirable qualifications of a sampling instrument included (1) accuracy over the spectrum of sizes characteristic of air-borne uranium dusts, (2) standardization for reproducibility of results, (3) ability to handle dusts of high density, and (4) adaptability to large-scale routine analyses in both field and laboratory. Moreover, two problems were involved, the measurement of uranyl fluoride fume resulting from hydrolysis of uranium hexafluoride, and the measurement of uranium dusts of other compounds resulting from grinding and other operations. Since the particle size of the fume is below the limits of resolution of the optical microscope it can be adequately measured only with the electron microscope. Accordingly the search for adequate routine methods was concerned primarily with dust.

Three instruments were given consideration: (1) the air centrifuge,[35] (2) the photographic ultramicroscope,[36] and (3) the Cascade impactor.[37] The available information on the air centrifuge was found to be limited. Investigation revealed that the instrument would require considerable redesigning and development. The type of engineering problems was considered to be too extensive for the immediate needs of the problem. Photographic ultramicroscopy, probably one of the best methods for the general air-borne-dust problem, gives results that are reproducible and accurate but is primarily a research tool not easily adaptable to routine and field use. The Cascade impactor was selected as the most promising instrument to fulfill the requirements in providing a system capable of yielding accurate and reproducible results. At the same time the instrument had the possibility of being adapted for routine use by untrained technicians. It could also be adapted to standardized production in quantity.

(b) The British Cascade Impactor. (1) General Construction and Theory of Operation. The details of the construction and the operating characteristics of the British Cascade Impactor were made available to the group at Rochester through the Scientific Research and Development Section of the British Ministry of Supply and the Chemical Warfare Service of the U. S. Army. Several of these instruments were constructed, and tests on uranium dust made. Since published information is now available (May),[37] the specific details of construction and operating characteristics of the instrument will not be discussed. Some general considerations, however, will be made in view of modifications in the instrument as a result of the uranium-dust studies. Because of these modifications the original instrument will be referred to as the "British Cascade Impactor."

The British Cascade Impactor consists essentially of a system of four jets and sampling slides in series. For convenience the collecting tubes of each section are designated jets 1, 2, 3, and 4, and the orifices are separately designated as slits 1, 2, 3, and 4. Sampling or collecting slides are called stages A, B, C, and D, respectively. The general assembly of the instrument is given in Fig. 10.14.

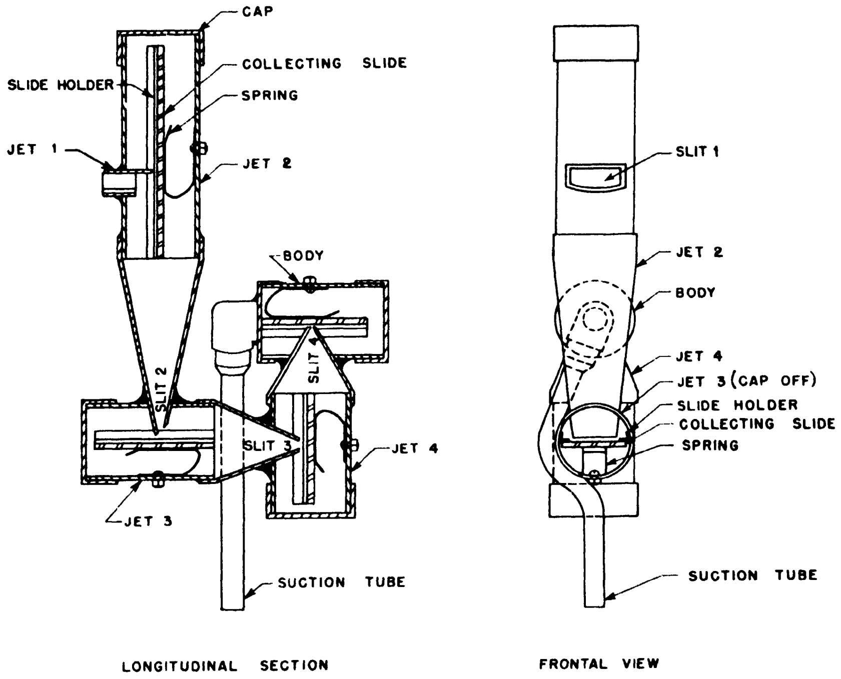

Fig. 10.14—Assembly drawing of the British Cascade Impactor.

The jets are progressively smaller, so that the speed and therefore the efficiency of impingement of particles on slides increases from stage to stage when dust-laden air is drawn through the instrument at a steady rate. The first stage tends to remove the largest particles, the second stage the largest of the remaining particles, etc. Thus the total distribution is broken into four overlapping distributions, one on each stage, each of which is a segment of the original distribution.

The individual distributions are measured with an optical microscope, and the over-all distribution is calculated by weighting these distributions against the total number of particles on each of the respective stages. The size-count distribution is then converted to a size-mass distribution by the procedure previously discussed. For a given distribution May suggests that the median sizes for stage B, C, and D are relatively constant, whereas those of stage A depend upon the upper limit of the distribution. If such is the case, then the total distribution can be characterized by simply analyzing the material on the stages chemically and correlating the results with the individual-stage mass distributions found. The method of mass distributions by chemical analysis promised an ideal solution to the practical problem of routine measurement of sizes.

The British Cascade Impactor had not previously been used with high-density small-size dusts, such as those of uranium compounds, but was designed for use with coarse aerosols of unit density and size range of from 1.5 to 50 μ. From theoretical considerations it seemed probable, however, that the high density of the uranium compounds would permit the instrument to handle the smaller sizes. Therefore, application of this instrument was made to four uranium dusts used in inhalation experiments. These included uranium dioxide, UO_2; uranium tetrafluoride, UF_4; uranium tetrachloride, UCl_4; and uranyl nitrate, $UO_2(NO_3)_2$; compounds of widely different chemical and physical properties. Preliminary studies were made with UO_2, the most stable of the dusts. These studies sought to obtain information directed toward improvement in the design and operation of the instrument for routine use. Because the application of the principle of multiple impaction to dusts presents unique problems, the detailed procedures and results obtained with the British instrument are given in the next section. For those interested only in general conclusions, the detailed discussion may be omitted.

(2) Use of the British Cascade Impactor with Uranium Dusts. i. General Procedure. Samples were collected from the uranium-dust-exposure units through a standard sampling port at approximately mid-height in the side wall of each unit. The impactor was inserted 18 in. into the units, with the axis of the opening of the first jet held horizontally with reference to the chamber floor. The sampling rate in all cases was 16.9 liters/min. Samples were collected on glass slides coated with a thin layer of trap adhesive as suggested by May.[37] The most suitable material for this purpose was found to be a nondrying alkyd resin.* A 50 per cent solution of the resin in

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*Alkyd Resin (5519-132), supplied through the courtesy of Dr. F. C. McGrew of E. I. du Pont de Nemours & Company.

xylene gave excellent optical surfaces at all microscopic magnifications and remained sticky for periods of several days. Particles collected on the resin are partially or fully embedded in the matrix. For microscopic examination a cover slip was placed on the top of the resin. The resultant air space between the cover slip and the resin coat was equivalent to an air mount. An attempt to eliminate this air space by the addition of an extra drop of resin was unsuccessful because of streaming effects and the disintegration of the dust streak.

Size measurements on collected samples were made with the projection microscope at a magnification of 5,000× on a grid calibrated at 1 μ intervals. Size-count and size-mass distributions were calculated for each stage. These results were then integrated to determine the total dust distribution. The mass medians obtained for each stage were tested for constancy to determine the validity of the method of chemical analysis.

ii. Particle-size Distribution of UO_2 Obtained by Optical Measurement. A 5-min dust sample at a concentration of 1 mg of UO_2/cu m was drawn from the chamber. Microscopic examination indicated typical UO_2 dust with apparently a minimal amount of contaminating material, although, at the resolution limits, atmospheric nuisance dust could not be differentiated.

The size-count distributions were determined for each of the impactor stages and represent a total of 4,500 size measurements. Distribution values are shown in Table 10.3. Total size-count distributions were obtained from individual stage distributions by weighting the values at each size limit in terms of the number proportions of particles found on each stage. In order to obtain the number proportions on individual stages, a total count was made of the numbers of particles in a strip 20 μ wide across the narrow dimension of the streak. Table 10.4 shows the averages of a series of 10 such counts made on each sample.

The total size-mass distribution was calculated from the total size-count distribution values by utilizing the cube of the average diameter (Table 10.3) as previously mentioned. The various size distributions for both the count and the mass functions are shown plotted on log-probability grids in Figs. 10.15 and 10.16, respectively. In all cases standard procedure was followed in which, ignoring the extremes of the distribution, the best straight line fitted through the points represented the distribution. The median values and the standard deviations, σ_g, were determined directly from these curves. A median size for the total size-count distribution of UO_2 was 0.95 μ with a standard deviation of 2.5. The median value for the total size-mass distribution was 5.3 μ, also with a standard deviation of 2.5. This agreement of values for the standard deviation is expected from theoretical considerations.

Table 10.3 — Size Distribution of UO_2 Dust Obtained with the British Cascade Impactor

	Count distribution, % less than upper limit					Mass distribution, % less than upper limit				
Limit size, μ	Stage A	Stage B	Stage C	Stage D	Total sample	Stage A	Stage B	Stage C	Stage D	Total sample
1.0	20.2	39.1	54.4	76.0	50.1	0.14	1.8	6.8	19.4	2.3
2.0	57.4	70.8	89.7	95.9	81.0	2.2	14.2	42.4	59.4	13.7
3.0	74.4	89.4	97.1	99.0	92.6	5.5	38.4	67.7	80.8	27.8
4.0	79.4	96.2	99.1	99.8	96.6	7.7	59.4	83.2	93.5	39.6
5.0	85.2	98.2	99.6	100	98.0	12.8	71.5	91.6	100	47.6
6.0	88.2	99.0	99.8		98.6	17.3	79.9	96.8		53.3
7.0	92.2	99.7	100		99.2	27.0	91.4	100		62.8
8.0	94.8	99.9			99.45	36.7	96.3			68.9
9.0	95.8	100			99.56	41.5	100			72.6
10.0	97.0				99.65	50.0				76.6
11.0	97.2				99.66	51.8				77.3
12.0	97.8				99.70	59.2				80.5
13.0	99.2				99.80	80.9				90.6
14.0	99.5				99.83	88.6				94.3
15.0										
16.0	100				100	100				100
17.0										

Table 10.4 — Size Distribution of UO_2 Dusts Obtained with the British Cascade Impactor

		Count distribution		Mass distribution	
Stage	Relative no. of particles	M_g, μ	σ_g	M_g, μ	σ_g
A	21.0	2.05	2.4	10.3	1.9
B	116.0	1.22	2.0	3.65	1.8
C	86.5	0.90	1.9	2.30	1.8
D	79.8	0.63	2.0	1.75	1.8
Total distribution		0.95	2.5	5.3	2.5

The median values and their standard deviations for both the count and mass distributions for the individual stages given in Table 10.4 show that satisfactory separation of median sizes and size ranges was obtained and indicates the practicability of the impactor for analysis of UO_2 size distribution by chemical methods. The plots of the

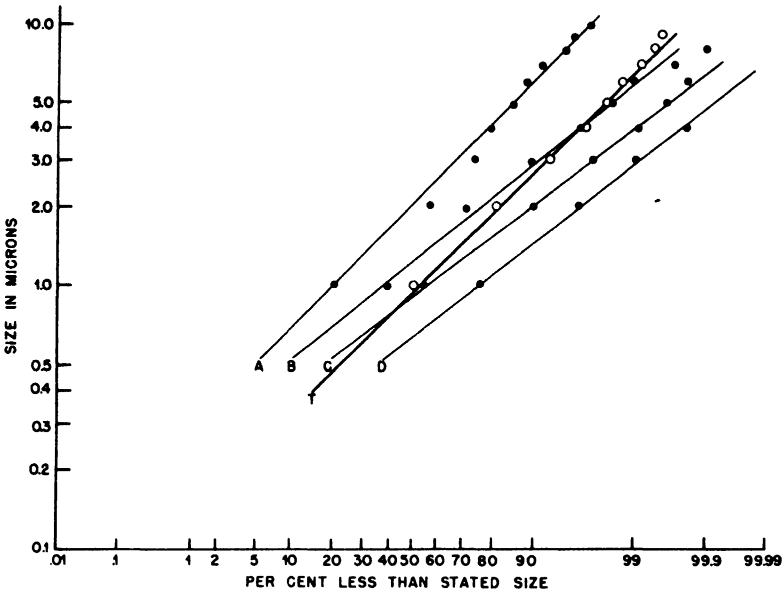

Fig. 10.15—Calibration of the British Cascade Impactor. Lines A, B, C, and D represent the size-count distribution of UO_2 dust obtained on each of the respective stages. The curve marked T represents the total size-count distribution obtained.

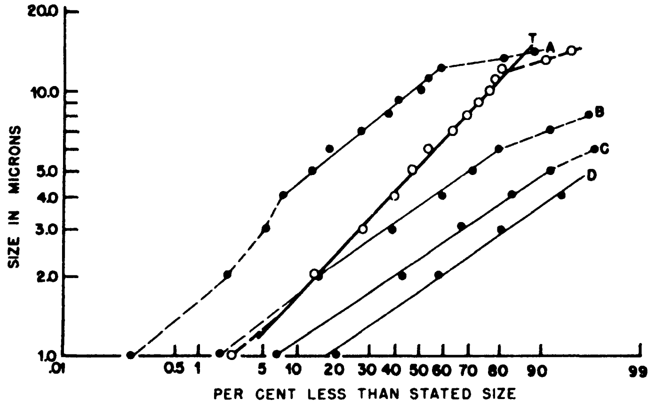

Fig. 10.16—Calibration of the British Cascade Impactor. Curves A, B, C, and D represent the size-mass distribution of UO_2 dust obtained on each of the respective stages. The curve marked T represents the total size-mass distribution obtained.

size and mass distributions for individual stages (Figs. 10.15 and 10.16) are in excellent agreement with other results reported for the British Cascade Impactor on materials of unit density. The agreement involves the closeness of fit of the normal curve to the general position of the actual plotted points and the divergence of the actual points at the extremes. On the log-probability plot, the curves for the size-count and size-mass distributions for all four stages are essentially parallel. The parallelism is shown more specifically in the values of the standard deviations obtained from these plots. For the count distribution the values were 2.0, 1.9, and 2.0 for stages B, C, and D, respectively, whereas that for stage A was 2.4. For the mass distributions the values are even better, with standard deviations of 1.8 obtained on each of the B, C, and D stages. The standard deviation value of 1.9 for stage A was only slightly different from that of the others. The parallelism obtained implies that a continuous distribution is sampled by the instrument and that mass distributions of the dust on each stage may be used as a basis for size analysis by chemical methods.

On the basis of the above parallelism it is permissible to use the mass median values as calibration constants of the impactor stages. The values 3.65, 2.30, and 1.75 for stages B, C, and D, respectively, were used for the calculation of the total size-mass distribution of UO_2 from chemically analyzed samples. The corresponding value for stage A is not generally used as a calibration constant, since it is a variable dependent upon the upper limits of the sample atmosphere.

iii. Particle-size Distribution of UO_2 by Chemical Analysis. The samples for the determination of mass distribution of UO_2 dust by the chemical method were collected on resin-coated slides. Sufficient material was collected on each of the stages to meet the minimal requirements of the ferrocyanide method of uranium analysis (Chap. 2). The data obtained for a set of seven samples are shown in Table 10.5. The size-mass distribution of stage A of each sample was first determined, however, by size measurement. The values obtained showed only minor variations, and therefore the average (10.6 μ) could be used as a calibration constant. Following standard procedure the results for each sample are shown in terms of percentage of UO_2, and also as a cumulative percentage of mass below the median size representing each stage. A good separation of material on each of the stages was found. The results were also consistent for a given stage, with only small variations. The average cumulative percentage values for the set of seven samples were stage A, 80.2 per cent; stage B, 43.3 per cent; stage C, 17.6 per cent; and stage D, 4.9 per cent.

Table 10.5 — Size Distribution of UO_2 Dust Obtained with the British Cascade Impactor and Determined by Chemical Analysis

Concentration level of compound, mg/cu m	Median size values, stage A, μ	Chemical analysis* of dust on stage					Mass distribution	
			A	B	C	D	Median, μ	σ_g
1	10.5	Mg UO_2	0.162	0.189	0.064	0.030	5.2	2.2
		% UO_2 total	36.5	42.5	14.3	6.7		
		Cumulative %	82.0	42.3	13.9	3.4		
	10.5	Mg UO_2	0.116	0.088	0.045	0.034	5.0	2.5
		% UO_2 total	41.0	31.1	15.9	12.0		
		Cumulative %	79.5	43.5	19.9	6.0		
10	10.5	Mg UO_2	0.057	0.042	0.037	0.015	4.7	2.5
		% UO_2 total	37.7	27.8	24.5	9.9		
		Cumulative %	81.1	48.3	22.2	5.0		
	10.5	Mg UO_2	0.349	0.277	0.145	0.068	5.3	2.5
		% UO_2 total	41.6	33.0	17.3	8.0		
		Cumulative %	79.2	41.9	16.8	4.1		
	11.1	Mg UO_2	0.392	0.299	0.116	0.068	5.9	2.5
		% UO_2 total	45.8	32.6	13.6	8.0		
		Cumulative %	77.1	37.9	14.8	4.0		
	10.6	Mg UO_2	0.348	0.292	0.120	0.068	5.4	2.4
		% UO_2 total	42.0	35.2	14.5	8.2		
		Cumulative %	78.9	40.3	15.5	4.1		
	10.3	Mg UO_2	0.250	0.295	0.136	0.086	4.6	2.4
		% UO_2 total	32.7	36.8	17.7	11.1		
		Cumulative %	83.8	48.1	20.0	5.6		
Average values	10.6	Cumulative %	80.2	43.3	17.6	4.9	5.2	2.4

* Mg UO_2 = amount of UO_2 dust in milligrams determined by chemical analysis of each stage.

% UO_2 total = per cent of UO_2 dust on each stage relative to total collected on all stages.

Cumulative % = successively summed per cent of UO_2 dust concentration at each stage, starting with stage D.

When the cumulative percentage values are plotted on a log-probability grid against the median mass sizes of each of the stages, a straight line is not obtained, indicating that the distribution was not normal. Three examples are given in Figs. 10.17B, C, and D, represented by the broken lines. However, the distributions sampled for chemical analysis given in Table 10.12 are taken from the same atmosphere and by the same instrument as those taken for calibration by optical measurement (Fig. 10.17A); therefore the results of chemical analysis must be a straight line. The best approximation to the distribution obtained by optical measurement (Fig. 10.17A) is the straight line drawn between points for stage A and C.

The intercepts of the cumulative percentage values obtained by chemical analysis on the ordinate (size scale) of the optically measured distribution curve (Fig. 10.17A) are more accurate constants.

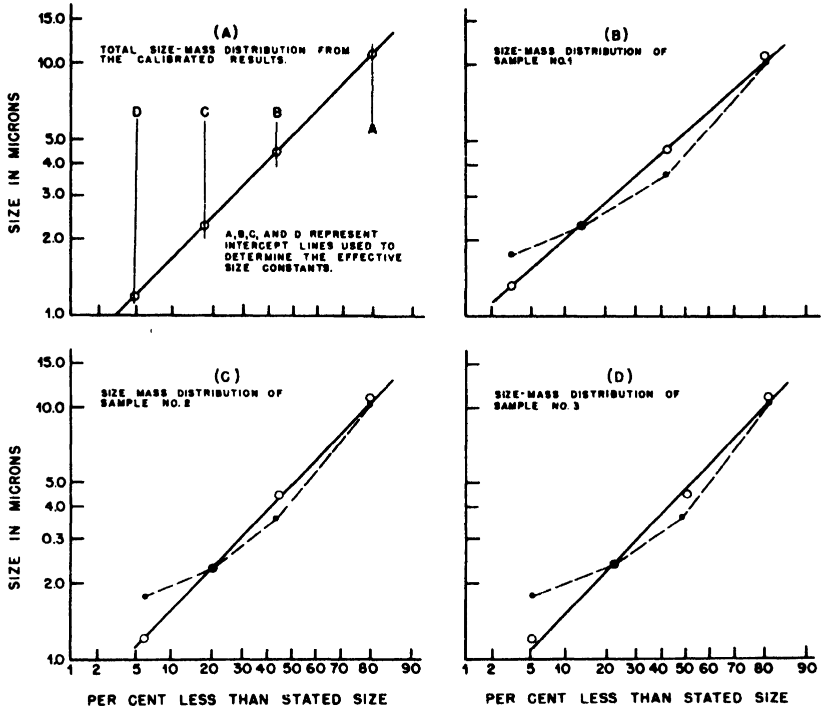

Fig. 10.17—Size-mass distributions of UO_2 obtained with the British Cascade Impactor. (Results obtained by the chemical-analysis method.) Curves drawn through points marked by a circle represent the distributions obtained by use of the effective-size values as calibration constants. These are determined from the mass intercepts shown in (A). Curves drawn through solid points represent the distribution shown by use of stage median values as calibration constants.

These are designated "effective-size constants." Figure 10.17A shows the intercepts as points marked by a circle. The effective-size constants obtained were: stage A, 10.9 μ; stage B, 4.5 μ; stage C, 2.29 μ; and stage D, 1.20 μ.

The distribution obtained by plotting the effective-size constants against the cumulative percentage values resulted in a straight line in all the samples studied (illustrated in Figs. 10.17B, C, and D, solid line plotted through point enclosed with a circle), indicating a normal distribution. The size-mass medians obtained in this way on the 1- and 10-mg levels of UO_2 (Table 10.5) varied from 4.6 to 5.9 μ with an average of 5.2 μ, which was in excellent agreement with the optically measured value of 5.2 μ previously obtained. Similar agreement was found for the standard deviations with an average of 2.4 compared to a previously measured value of 2.5. Therefore the chemical method of determining size-mass distribution was considered practicable for uranium dusts.

iv. British Cascade Impactor Results with Other Uranium Compounds. Calibration tests of the British Cascade Impactor were also made with dusts of uranyl nitrate, uranium tetrachloride, and uranium tetrafluoride. Each compound showed good separation of particle size and of mass concentration on the individual impactor stages. However, certain factors, which included specific properties of the compounds tested as well as a serious interference of atmospheric nuisance dust, made difficult the determination of total count and total mass-distribution values by microscopic count, although approximate determination of the distribution of individual stages was possible. Values for each stage were determined by neglecting nuisance dust and other material that appeared obviously atypical. The total distributions determined by the chemical-analysis method were taken as a normal curve paralleling that found for uranium dioxide.

v. Uranyl Nitrate. Microscopic examination of the material on each of the stages showed the uranyl nitrate dust to be extremely hygroscopic. Many irregular masses of wet crystalline material were found on stage A. There was evidence of much shattering and recrystallization. Stages B, C, and D showed many spherical forms having an appearance typical of liquid droplets. A 10-min sample at a level of 2 mg/cu m was used for the microscopic measurements. The mass median values, which were used as calibration constants for this dust, ranged from 0.86 μ for stage D to 15.2 μ for stage A. A good separation of the size groups was obtained (Table 10.6). Parallelism of the stage distributions is shown in the similarity of the standard deviations of 1.6 for stages A, B, and D, and 1.5 for stage C. A 1-hr sample from the same atmosphere was used for chemical analysis, the results of which also appear in Table 10.6. Good separation of the nitrate on each of the stages was obtained (54.2 per cent on stage A, 25.4 per cent on stage B, 11.8 per cent on stage C, and 8.5 per cent on stage D). The total mass-distribution curve determined from these values showed a median mass-size value of 7.5 μ with a standard deviation of 2.9.

Table 10.6 — Size-Mass Distribution of Uranyl Nitrate Dust Obtained with the British Cascade Impactor

	Calibrated size-mass distributions		Chemical analysis of the stages		
Stage	Size-mass medians, μ	Standard deviation, σ_g	Uranium conc., mg	Uranium, %	Cumulative percentage
A	15.2	1.6	0.32	54.2	72.9
B	3.5	1.6	0.15	25.4	33.1
C	2.48	1.5	0.07	11.8	14.5
D	0.86	1.6	0.05	8.5	4.3

Table 10.7 — Size-Mass Distribution of Uranium Tetrafluoride Dust Obtained with the British Cascade Impactor

	Calibrated size-mass distributions		Chemical analysis of the stages		
Stage	Size-mass medians, μ	Standard deviation, σ_g	Uranium conc., mg	Uranium, %	Cumulative percentage
A	7.7	1.7	0.69	59.5	70.3
B	5.0	1.6	0.26	22.4	29.3
C	2.1	1.8	0.14	12.1	12.1
D	1.24	1.9	0.07	6.0	3.0

vi. Uranium Tetrafluoride. Microscopic examination of dust samples of the tetrafluoride showed that most of the material consisted of extremely thin, transparent crystals. Because of the transparency of the particles and the similarity of the refractive indices of the material to that of the collecting resin, the resolution was very poor. A 10-min sample at a 1-mg/cu m level resulted in a high proportion of nuisance dust in the sample that introduced a serious problem in the measurements. The mass median sizes used as calibration constants are shown in Table 10.7. The spread of the medians from 1.24 μ for stage D to 7.7 μ for stage A shows again good separation of the size groups. On the other hand, parallelism of the distributions on the stages was not so good as in former instances as shown by the standard-deviation values ranging from 1.6 to 1.9. From a 75-min sample at the 1-mg level, a good separation of the material was obtained by chemical analysis (59.5 per cent on stage A, 22.4 per cent on stage B, 12.1 per cent on stage C, and 6.0 per cent on stage D, Table 10.7). The mass median size for the total distribution of uranium tetrafluoride was determined as 5.1 μ; the standard deviation was calculated as 2.1.

vii. Uranium Tetrachloride. At the low level available for optical measurement (0.2 mg/cu m) for which a 45-min sample was required, nuisance-dust concentrations were larger than those of uranium dust. The presence of several hydrolytic products and the extreme hygroscopicity of the chloride made precise measurements difficult. In addition the similarity of the refractive index of the material to that of the collecting resin resulted in extremely poor resolution. The calibration constants for each stage showed a spread of from 3.27 μ for stage D to 13.3 μ for stage A, a reasonably good separation (Table 10.8). A 1-hr sampling period, used for chemical analysis, showed that separation of material on each of the stages was good with 6.76 per cent on stage A, 58.2 per cent on stage B, 23.0 per cent on stage C, and 12.2 per cent on stage D (Table 10.8). The mass median size for the total distribution was 6.4 μ with a standard deviation of 1.45.

Table 10.8 — Size-Mass Distribution of Uranium Tetrachloride Dust Obtained with the British Cascade Impactor

	Calibrated size-mass distributions		Chemical analysis of the stages		
Stage	Size-mass medians, μ	Standard deviation, σ_g	Uranium conc., mg	Uranium, %	Cumulative percentage
A	13.3	2.4	0.005	6.76	97.8
B	5.3	1.9	0.043	58.2	64.8
C	5.0	2.1	0.017	23.0	23.7
D	3.27	1.7	0.009	12.2	6.1

(3) Conclusions Regarding the British Cascade Impactor for Routine Use with Uranium Dusts. The results obtained with the British Cascade Impactor indicated that this instrument with certain modifications in design could be used to measure the size-mass distribution of uranium dust by the simple expedient of chemically analyzing the quantity of material deposited on each stage. It was first necessary, however, to overcome certain problems in instrument design and in the procedure of calibration and in method of optical measurement.

i. Problems of Design. The sheet-metal construction of the British instrument did not permit the orifices to be made with the degree of precision required and made difficult the duplication of instruments.

The collected streaks were irregular and the numbers of particles deposited were uneven, introducing errors in the determination of total count per slide. The impactor was not of rugged construction and was easily damaged under conditions of continual use. The end caps became loose, and difficulty was experienced in making an airtight seal. The lack of a smooth-walled interior, a consequence of its construction, resulted in high internal losses due to wall impingement of the order of 15 to 50 per cent of the total sampled dust. Because the stages were soldered together, it was impossible to determine losses in each jet, and thus no correction factor could be applied. Some of the smaller particles passed completely through the impactor without impinging on any of the stages. This amounted to approximately 10 per cent of the total material sampled.

To overcome these deficiencies a Modified Cascade Impactor (Sec. 8.2d) was developed, making liberal use of the principles of design of the British instrument.

ii. Problems in Calibration. Calibration of the instrument involved two problems: nuisance dust and the methods of chemical analysis. To determine the amount of nuisance dust that might be expected to interfere at low uranium-dust concentrations several 1-cu m air samples from the exposure units* were taken with the British Cascade Impactor during the animal conditioning period when no uranium dust was present. The quantities of nuisance dust removed from the air were sufficiently large to show a clearly visible streak on each stage on microscopic examination. Most of the material on the slides appeared to be organic in nature. It stained readily with Wright's stain and had the appearance of thick flaky section. Sizes ranged from 1 to 3 μ on the largest axis. Although no identifiable animal structures were seen, and only scant amounts of animal matter such as hair were found, it was concluded that the collected material was largely of plant origin. Similar structures have been observed in sawdust and wood shavings. Spectrographic analysis of such samples revealed these elements in quantities of roughly from 1 to 2 μg/cu m: Pb, Cu, Mo, Ag, Fe, Al, Si, Bi, Mg.

A series of dust counts in terms of numbers of particles per cubic foot of air was made with a Bausch & Lomb dust counter of both room air and exposure-chamber air. Results in Table 10.9 show average room count to be 0.76×10^6 particles per cubic foot, which is much lower than the average value of 1.9×10^6 particles per cubic foot given by the U. S. Public Health Service[38] for general room atmospheres. The difference resulted from the use of air-conditioning equipment in the exposure units. The UF_4 unit with no uranium dust but loaded with animals showed a slightly higher value of 0.96×10^6 particles per cubic foot. All other values given for units with uranium-dust atmospheres were below the average room-count values with the exception of the 10-mg level of UO_2, which had a count of 4.2×10^6 particles per cubic foot.

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* Unit used for the 1-year inhalation studies.

Table 10.9 — Dust Counts of Room and Exposure-unit Atmospheres

Area sampled	Compound	Concentration level of compound, mg/cu m	Dust count, particles per cu ft × 10^6			
			1	2	3	Average count
Room atmosphere			0.5	0.5	1.3	0.76
UF_4 exposure unit conditioning period	No uranium dust	0.0	0.3	1.7	0.9	0.96
Uranyl nitrate exposure unit	Uranyl nitrate hexahydrate	2.0	0.4	0.9	0.7	0.66
Uranyl nitrate exposure unit	Uranyl nitrate hexahydrate	0.3	0.5	0.3	0.5	0.43
UO_2 exposure unit	UO_2	10	6.0	5.2	1.4	4.2
UO_2 exposure unit	UO_2	1	0.2	0.3		0.25

The presence of relatively large quantities of nuisance dust at the low uranium concentrations used in inhalation experiments was shown to interfere seriously with the optical measurement of particle size. Consequently, determination of the mass constants had to be made on a special atmosphere of high uranium-dust concentration in which there was negligible nuisance dust. The specific procedure is discussed in a later section.

The contamination by nuisance dust also interfered with the ferrocyanide method of analysis (Chap. 2). The fluorophotometric method (Chap. 2) was, therefore, adopted because it was least affected by contamination, and it also had the required sensitivity.

iii. Problems of Measurement. Size measurements and counts of the particles on resin-coated impactor slides were not satisfactory when examined by the projection microscope. The use of air mounts required for examination resulted in poor visibility and low resolution, as previously pointed out.

In order to calibrate the British Cascade Impactor satisfactorily, it was imperative to develop methods for accurate measurement of particle sizes. These methods comprised the use of a medium of very high refractive index in conjunction with an apochromatic microscope and the electron microscope. At the time this was written, the work with the electron microscope had not been completed, and therefore the present discussion is limited to the optical method.

(c) The Selenium-coating Technique for High-refractive-index Mounting. A series of studies on uranium dusts showed that the particle visibility requirements of the problem could best be met through the use of a mounting medium possessing a refractive index greater than 2. Although such media as realgar and selensulfur melts were available, they were not satisfactory for the present purpose because of the lack of purity and optical clarity. In 1939 Hanna and Grant[39] developed a technique of obtaining clear melts of realgar by the use of a vacuum-evaporation method. This technique was modified by Shillaber,[33] who used selenium metal. This element has a refractive index of 2.8 and possesses good optical quality. Selenium has, moreover, other advantages. In addition to the increased visibility it affords, glare and other objectionable features of air mounts are eliminated, and field depth is increased approaching maximal practical values. The resolution of the microscope is sufficiently increased so that the only limiting factors are those of the objective aperture and the index of refraction of the immersion oil required for the objective. In view of these unquestioned advantages, the selenium-coating technique was, therefore, adopted for use in size measurement.

A vacuum-evaporation apparatus was designed and constructed. Basically it consists of a system in which a film is produced on the specimen by evaporation from a filament in a high vacuum. This is essentially the apparatus commonly used for the production of front-surface aluminum mirrors. A discussion of the construction details and the problems encountered in high-vacuum work may be found in the literature.[40] The details of the system as developed for the uranium-dust problem are, however, illustrated diagrammatically in Fig. 10.18.

(1) Details of Construction of Vacuum-evaporation Apparatus. The evaporation chamber consists of an 18-in. glass bell jar and a metal base plate. The base plate is constructed from ¾-in. brass to measure 12 in. in diameter on which two insulated filament terminals are mounted. Adjustable filament supports are attached to these in the manner illustrated. The filament consists of 16 turns of 20-mil tungsten wire wound in the form of an inverted cone measuring ½-in. in height and ¼-in. at the base. Current is supplied to the filament from a 110-volt a-c line through a step-down transformer controlled by a Varitran transformer. The specimen holder is designed to hold a complete set of impactor slides directly above the filament. The filament-to-specimen distance is adjustable by means of a sliding

support that can be clamped on a supporting rod. The supporting rod is fixed in position by means of an adjustable clamp attached to one of the filament terminals. Normally, its position is 10 cm from the slides.

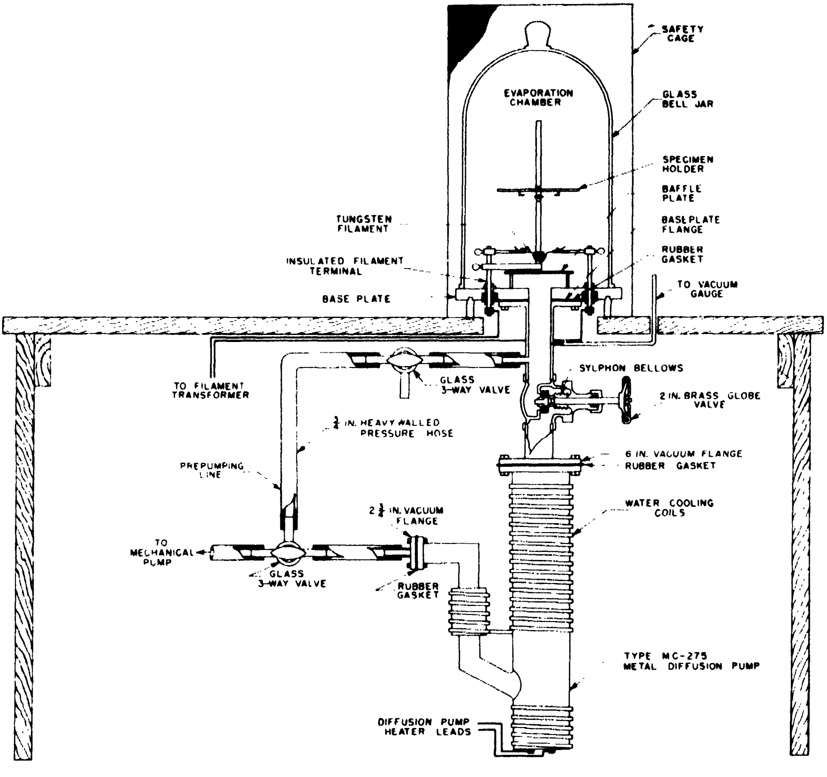


Fig. 10.18—Vacuum-evaporation apparatus.

The vacuum system consists of a Cenco Megavac pump, as a fore pump, and a Distillation Products MC 275 metal diffusion pump. The diffusion pump is connected to the evaporation chamber through a 2-in. brass line and to the fore pump through ¾-in. heavy-wall rubber pressure hose. A 2-in. brass globe valve is placed in the line between the evaporation chamber and the diffusion pump to permit closing of the pumping system from the evaporation chamber. The use of this valve for high-vacuum work required rebuilding in which the seat of the valve was reground and a sylphon bellows used to seal the inner valve stem and the bonnet. The three-way glass valve introduced between the diffusion pump and the fore pump serves to disconnect these systems and also provide a prepumping connection for the evaporation chamber. This connection is made through ¾-in. hose directly into the brass line leading into the evaporation chamber. A second three-way glass valve was introduced in the prepumping line to permit closing the entire line from the high-vacuum system. The extra arm of this valve serves as a bleeder enabling the evaporation chamber to be opened to the atmosphere after the coating has been completed. The pressures developed within the evaporation chamber were measured with both Pirani- and the ionization-type gauges through a connection in the line leading to this chamber.

(2) Operation. The filament is filled with small chips of selenium. The slides containing the dust samples are inserted in the specimen holder, and the bell jar is placed in position on the base. A vacuum seal between the jar and the base is made with Apiezon M grease. From a cold start the system is evacuated through both pumping systems without using the prepumping line. Pressures below 10^{-4} mm are obtained within 20 min. In repeated operations this time can be considerably shortened by keeping both pumps running and making use of the prepumping line. When the desired vacuum of 10^{-4} mm has been reached, sufficient time must be allowed to permit complete degassing of the sample. This depends upon the nature of the material and varies from several minutes to an hour or more. In order to coat the sample, a current of 20 amp is applied to the filament. Evaporation of the selenium is complete within 30 to 45 sec.

(3) Results. Excellent selenium coats were obtained on uranium-dust samples collected on clean, dry slides. The coatings examined under highest microscope magnifications have a red color but are perfectly transparent and show no signs of structure or impurities. The effect of the high refractive index of this film material is strikingly apparent in the severe contrast shown between the particles and the background and in the sharp particle details. The amount of material deposited is not critical but must possess as a lower limit a thickness of at least half a wave length; as an upper limit, the coat should not be so thick as to obscure visibility by the deepness of the red color. The thickness of the coat can be controlled by variation of several factors, namely, the filament-to-specimen distance, the amount of material placed within the filament, and the time allowed for evaporation. If necessary the coating procedure may also be repeated several times without harm to the specimen. The films are rather hard and stable under ordinary conditions. They are, however, not heat stable and were found to break down rapidly under conditions of

intense illumination necessary for a projection microscope. Used with a standard microscope and ordinary illumination, no difficulties were encountered.

A study of the pressures required to produce satisfactory coats of selenium metal showed that although the best coats were obtained with pressures below 10^{-4} mm, satisfactory coats could be obtained with pressures as high as 10^{-2} mm. Since this latter value is in the range of many of the ordinary types of high-vacuum pumps, a simple pumping system similar to that described by Hanna and Grant[39] may be of value as a routine instrument.

(4) The Effect of Selenium Coating on Particle-size Measurement. A study of the effect of selenium coating on particle-size measurements was made on a set of samples of UF_4 dust at a concentration of 1 mg/cu m. The samples were collected on clean, dry slides with a single-stage impactor. Dimensions of the instrument correspond to those of the tube and the slit of stage C of the British Cascade Impactor.

In order to permit a more detailed examination of the results obtained than possible with the group classification method, use was made of a Bausch & Lomb filar micrometer. This measuring device was fitted to the microscope and calibrated in conjunction with a 2-mm 1.30 numerical aperture (N.A.) apochromatic objective to read directly in intervals of 0.1 μ.

Particle-size measurements were first made on the samples using the conventional method of air mounting. Then the samples were coated with selenium and approximately the same area on each sample remeasured. Table 10.10 and Fig. 10.19 indicate that the count distribution shifts toward smaller sizes on selenium-coated slides. At the 1 μ limit a difference of 33.5 per cent in the number of particles was found between the coated and uncoated samples. This difference increased to 61 per cent at the 0.5 μ limit. The median size for the coated sample was only 0.25 μ, compared to a value of 0.89 μ for the uncoated sample, a decrease in median size of 71.9 per cent. The standard deviation for the coated distribution was 2.8 as compared to 1.7 for the distribution of the uncoated sample, indicating the extended range of sizes in the coated sample.

The mass-distribution results given in Table 10.11 also show the same shift toward smaller sizes on the coated slides as compared to uncoated slides. At the 1-μ limit a difference in the mass of 9.8 per cent was found between the coated and uncoated sample. This shift is not so marked as in the case of the count distribution. This is to be expected, since the mass represented by size groups is influenced to a greater extent by the larger sizes than by the smaller. The mass

Table 10.10—Comparison of Size-Count Distribution of Uncoated and Selenium-coated UF_4 Dust Particles

	Uncoated			Selenium-coated		
Size, μ	No.	No. less than upper limit	% less than upper limit	No.	No. less than upper limit	% less than upper limit
0.1	1	1	0.5	9	9	4.5
0.2	1	2	1.0	38	47	23.5
0.3	5	7	3.5	39	86	43.0
0.4	11	18	9.0	36	122	61.0
0.5	8	26	13.0	26	148	74.0
0.6	18	44	22.0	15	163	81.5
0.7	21	65	32.5	7	170	85.0
0.8	19	84	42.0	7	177	88.5
0.9	16	100	50.0	4	181	90.5
1.0	20	120	60.0	6	187	93.5
1.1	10	130	65.0	0		
1.2	18	148	74.0	2	189	94.5
1.3	5	153	76.5	1	190	95.0
1.4	8	161	80.5	1	191	95.5
1.5	7	168	84.0	2	193	96.5
1.6	3	171	85.5	2	195	97.5
1.7	3	174	87.0	0		
1.8	3	177	88.5	0		
1.9	1	178	89.0	0		
2.0	4	182	91.0	1	196	98.0
2.1	2	184	92.0	0		
2.2	3	187	93.5	0		
2.3	3	190	95.0	0		
2.4	1	191	95.5	0		
2.5	0			1	197	98.5
2.6	0			0		
2.7	1	192	96.0	1	198	99.0
2.8	0			1	199	99.5
2.9	0			0		
3.0	1	193	96.5	0		
3.2	1	194	97.0	0		
3.4	1	195	97.5	0		
3.5	1	196	98.0	0		
3.7	1	197	98.5	0		
3.8	0			1	200	100
4.3	0			0		
4.9	1	198	99.0	0		
5.4	1	199	99.5	0		
5.7	1	200	100	0		

median size for the coated sample was found to be 3.0 μ, a significant decrease of 16.7 per cent from the value of 3.6 μ for the untreated sample. The standard deviation for the coated distribution was 2.73 as compared to 2.28 for uncoated slides.

The results decisively show the value of coating dust samples with a medium of high refractive index. Since the major portions of the dust distribution measured using the coating technique were below the resolution limits of the ordinary methods of measurement, it can be concluded that size distributions obtained previously by optical methods alone require reexamination.

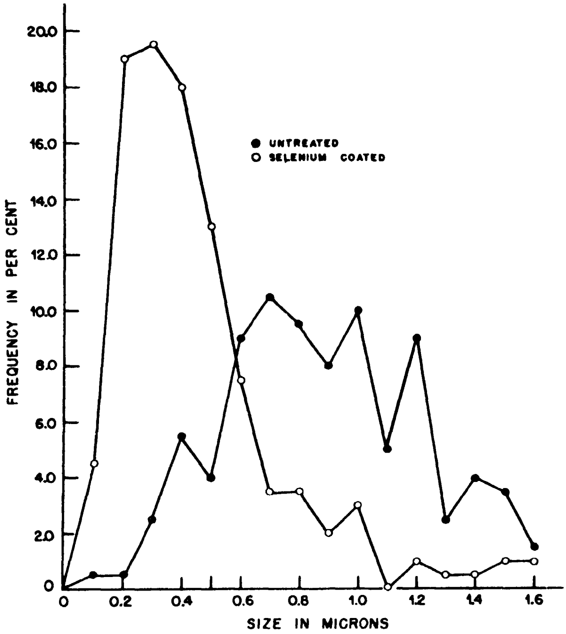


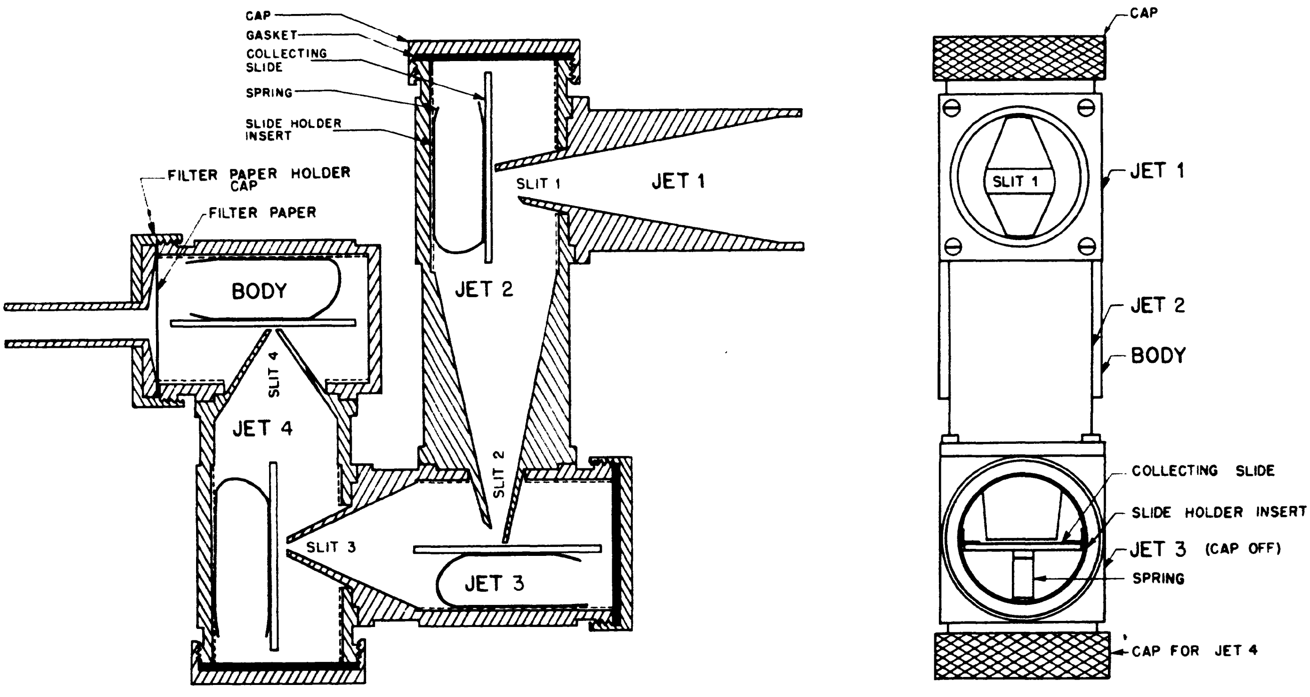
Fig. 10.19—Effect of selenium coating on particle-size measurement. Size-count distribution of a UF_4 dust sample.

As early as 1923, Martin[28] and coworkers, in a study on fine grinding, showed that the mode of the distribution shifted to smaller and smaller sizes as the magnifying power of the microscope increased and that the observed distributions were dependent on the type of measuring equipment. The recent development of the electron microscope and its application to the problem of particle-size measurements have added a considerable amount of evidence to the thesis that size groups are to be found in significant numbers below the resolution limits of the ordinary microscope. Results demonstrated by Brubaker[41] showed that size determinations on commercial zinc oxides required revision of from 25 to 50 per cent of the previous values obtained with the light microscope.

Table 10.11—Comparison of Size-Mass Distribution of Uncoated and Selenium-coated UF_4 Dust Samples

		Uncoated				Selenium coated			
Size, μ	Mass factor, D^3	No.	Weighted group, ND^3	ND^3 less than size limit	% less than size limit	No.	Weighted group, ND^3	ND^3 less than size limit	% less than size limit
0.1	0.001	1	0.001	0.001	0.00009	9	0.009	0.009	0.005
0.2	0.008	1	0.008	0.009	0.0008	38	0.304	0.313	0.185
0.3	0.027	5	0.135	0.144	0.014	38	1.026	1.339	0.795
0.4	0.064	11	0.704	0.848	0.083	36	2.304	3.643	2.16
0.5	0.125	8	1.000	1.848	0.183	26	3.250	6.893	4.08
0.6	0.216	18	3.888	5.736	0.566	15	3.240	10.13	6.00
0.7	0.343	21	7.203	12.94	1.27	7	2.401	12.53	7.43
0.8	0.512	19	9.728	22.67	2.24	7	3.584	16.11	9.57
0.9	0.729	16	11.724	34.39	3.39	4	2.916	19.02	11.2
1.0	1.00	20	20.0	54.39	5.37	6	6.00	25.02	14.8
1.1	1.331	10	13.31	67.70	6.69	0			
1.2	1.728	18	31.10	98.80	9.77	2	3.456	28.48	16.8
1.3	2.197	5	10.99	109.8	10.8	1	2.197	30.67	18.2
1.4	2.744	8	21.95	131.7	13.0	1	2.744	33.41	19.7
1.5	3.375	7	23.63	155.4	15.4	2	6.750	40.16	23.8
1.6	4.096	3	12.29	167.7	16.6	2	8.182	48.34	28.6
1.7	4.913	3	14.75	182.4	18.0	0			
1.8	5.832	3	17.50	199.9	19.8	0			
1.9	6.859	1	6.859	206.7	20.4	0			
2.0	8.00	4	32.00	238.7	23.6	1	8.00	56.34	33.2
2.1	9.261	2	18.52	257.2	24.3	0			
2.2	10.65	3	31.95	289.1	27.6	0			
2.3	13.17	3	36.51	325.6	32.2	0			
2.4	13.82	1	13.82	339.4	33.5	0			
2.5	15.63	0				1	15.63	71.97	42.7
2.6	17.58	0				0			
2.7	19.68	1	19.68	359.1	35.4	1	19.68	91.65	59.3
2.8	21.95	0				1	21.95	113.6	67.3
2.9	24.39	0				0			
3.0	27.00	1	27.00	386.1	38.2	0			
3.2	32.78	1	32.78	418.9	41.3	0			
3.4	39.30	1	39.30	458.2	45.2	0			
3.5	42.88	1	42.88	501.1	49.5	0			
3.7	50.65	1	50.65	551.8	54.5	0			
3.8	54.87	0				1	54.87	168.5	100
4.3	79.51	0				0			
4.9	117.6	1	117.6	669.4	66.1	0			
5.4	157.5	1	157.5	826.9	81.7	0			
5.7	185.2	1	185.2	1,012	100	0			

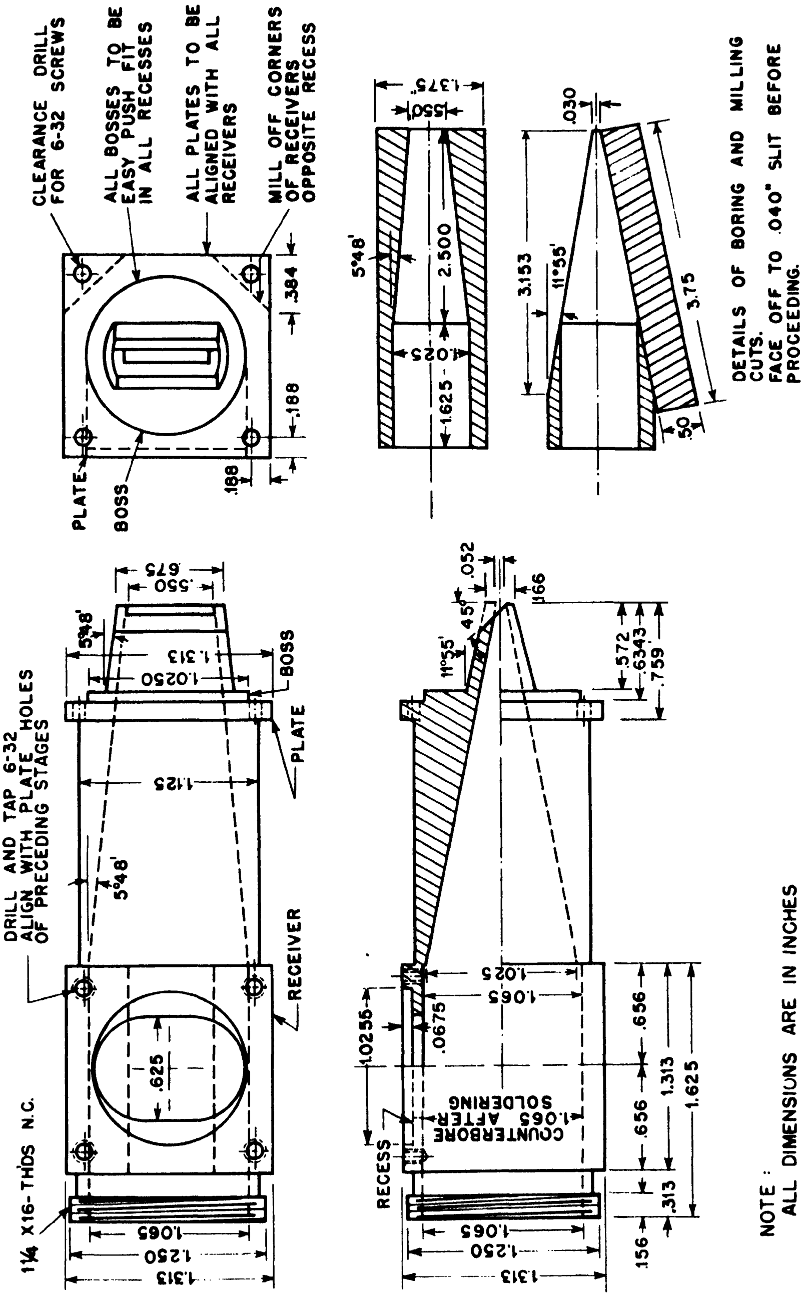
(d) The Modified Cascade Impactor. (1) Construction. On the basis of experience gained with the British Cascade Impactor an improved model was constructed to suit the requirements of the uranium dusts. This impactor was constructed of solid brass by precision machining methods rather than by working of sheet metal. The individual jets were constructed as independent interchangeable units. The general assembly of the complete instrument is shown in Fig. 10.20. Specific construction details of one individual jet are shown in Fig. 10.21, to illustrate the method of machining (specifications available at University of Rochester, Atomic Energy Project). The slits were accurately made and could be duplicated from instrument to instrument.



LONGITUDINAL SECTION

FRONTAL VIEW

Fig. 10.20—Assembly drawing of the Modified Cascade Impactor.


1¼ X16-THDS N.C.
DRILL AND TAP 6-32 ALIGN WITH PLATE HOLES OF PRECEDING STAGES
RECEIVER
PLATE
BOSS
CLEARANCE DRILL FOR 6-32 SCREWS
ALL BOSSES TO BE EASY PUSH FIT IN ALL RECESSES
ALL PLATES TO BE ALIGNED WITH ALL RECEIVERS
MILL OFF CORNERS OF RECEIVERS OPPOSITE RECESS
RECESS
COUNTERBORE 1.065 AFTER SOLDERING
1.313
1.250
1.065
.625
1.125
1.0250
.550
.675
5°48'
.188
.384
1.375"
.550
2.500
1.025
1.625
.030
3.153
11°55'
3.75
.50
.052
45°
.166
.572
.6343
.759
.10255
.0675
.656
.313
.156

DETAILS OF BORING AND MILLING CUTS.
FACE OFF TO .040" SLIT BEFORE PROCEEDING.

NOTE:
ALL DIMENSIONS ARE IN INCHES

Fig. 10.21—Construction details and specifications of jet 2 of the Modified Cascade Impactor.

The original slit dimensions and internal streamlining of the instrument were patterned after those of the British Cascade Impactor with the exception of jet 1, which was modified for the particular conditions. The machining method allowed for highly polished interiors reducing internal losses to a minimum. End losses, representing the portion of the dust distribution that passed through the British instrument, were collected on a filter paper that served as a fifth stage.

The instrument as a whole was of rigid, sturdy construction, which would stand the rigors of routine use. It was designed to permit mass production with accuracy limited only by the tolerances of standard machine operation. By modern die-casting methods, exact duplication in large numbers is possible. Uniformity between instruments is desirable because the calibration constants for one instrument are thus applicable to all others.

(2) Calibration Procedure, General. The new instrument was calibrated with uranium dusts making full use of the modifications in technique discussed previously, including the use of selenium as a mounting medium. For accurate sampling the impactor slides are coated with a resin that traps impacted particles. Unfortunately, selenium could not be applied to resin-coated slides. Although dry-collected slides (no resin) result in high losses from shattering of particles and failure of impingement, only such samples could be used with the selenium-coating technique. Accordingly it was necessary to adopt an indirect procedure to obtain a calibration equivalent to that for resin-collected slides measured with selenium coating. For this purpose a series of three calibrations was undertaken: I, with resin-collected slides measured as resin mounts; II, with dry-collected slides measured as resin mounts; and III, with dry-collected slides measured as selenium mounts. Methods I and II were consistent in so far as the method of measuring but differed in the method of sampling. Methods II and III were identical in the method of sampling but were optically different. From the data it was possible to correct method II by method III to make use of the more accurate measurements obtained with the high-refractive-index coating. In addition it was possible to correct method II by the results of method I in order to take advantage of the collecting efficiency of the resin coating. By such procedure accurate constants were obtained that could be applied to routine samples taken from animal chambers and analyzed for mass distribution by chemical methods. The constants obtained were in excellent agreement with those expected from theoretical considerations. Constants for one dust could also be used to calculate constants for other dusts of different densities.

From the results to date the Modified Cascade Impactor appears to be an excellent instrument for routine measurement of dust distribution and could be adopted as a standard instrument. The calibration procedure for this instrument is therefore given in considerable detail in the following section.

(3) Calibration Procedure of Modified Cascade Impactor. The compound UO_2 was used to calibrate the modified impactor because this dust presented fewer problems than did the other available uranium compounds. In order to avoid the nuisance-dust problem, a high UO_2 concentration was used. A small chamber of 0.5-cu m capacity, similar to those used for animal work, was operated at a concentration of 40 mg/cu m. The source of dust was identical with that used for animal-exposure units, and was designated as the "standard UO_2 atmosphere." A sampling period of from 1 to 2 min was used at a rate of 14.0 liters/min.

For measurement, a microscope equipped with a 2-mm apochromatic objective of numerical aperture 1.30, an aplanatic-achromatic condenser, and compensative eyepiece (25×) was used. Measurements were made with an ocular micrometer with a scale of 20 divisions per millimeter. It was calibrated to read directly in units of 0.5 μ. A size-classification method of measuring particles was used.[20]

Altogether 35 sets of impactor samples were taken of the standard UO_2 atmosphere. Fifteen of these sets were collected on resin alternated by twenty sets collected on dry slides. Of the 15 sets collected on resin, 10 were analyzed chemically; the remaining 5 were measured optically after the addition of a drop of resin and cover slip (method I). Of the 20 sets collected on dry slides, 10 were analyzed chemically, 5 were measured optically after the addition of a drop of resin and a cover slip (method II), and the other 5 were measured following coating with selenium (method III).

Two hundred particles were measured on each of stages A, B, C, and D. For each set of five optically measured samples, the 200 measurements per stage were grouped in order to achieve a representative sample, giving a total count of 1,000 particles per stage for each method. In order to demonstrate the specific procedure of calculating stage-mass distributions from the raw data, a representative set of tabulations (those for method I) is illustrated in Tables 10.12, 10.13, 10.14, and 10.15. The same procedure was used for methods II and III. Since stage E in each case is a filter paper, the distribution of this stage was calculated from theory as the most probable value.

(4) Total Size-Mass Distributions. These were obtained for each of the methods by weighting the mass frequency in percentage for each size class on each stage in terms of the average percentage mass

Table 10.12 — Size-Mass Distribution

Instrument: Modified Cascade Impactor
Stage: A
Method I: resin collected

Compound: UO_2
Area sampled: special unit
Sampling time: 1 min

Limit size, μ	No. frequency										ΣN	Average group size, μ	Mass factor, D^3	Weighted groups, $\Sigma N \times D^3$	Mass frequency, %	% less than size limit
	N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8	N_9	N_{10}						
0.5	37	42	19	19	0	5	7	5	20	17	181	0.25	0.0156	2.823	0.0101	0.0101
1.0	35	29	31	29	33	42	55	49	44	57	404	0.75	0.422	170.5	0.615	0.625
1.5	5	8	13	16	15	14	19	19	15	8	132	1.25	1.955	257.4	0.924	1.55
2.0	2	2	12	7	13	8	9	9	3	5	70	1.75	5.35	374.5	1.35	2.80
3.0	5	1	9	10	8	7	3	10	8	5	66	2.50	15.6	1,030	3.70	6.50
4.0	6	1	8	13	5	3	3	2	12	3	58	3.50	42.9	2,488	9.96	15.46
5.0	4	5	8	8	9	5	2	1	3	2	47	4.50	91.1	4,282	15.4	30.9
6.0	4	0	3	5	8	8	2	3	2	2	37	5.50	166	6,142	22.1	53.0
7.0	1	1	4	2	5	6	1	2	1	1	24	6.50	275	6,600	23.7	86.7
8.0	1	1	0	1	3	3	0	0	0	0	9	7.50	422	3,798	13.6	90.3
9.0	0	0	1	0	1	1	0	0	0	0	3	8.50	614	1,842	6.62	96.9
10.0	0	0	1	0	0	0	0	0	0	0	1	9.50	857	857	3.08	100

Table 10.13 — Size-Mass Distribution

Instrument: Modified Cascade Impactor
Stage: B
Method I: resin collected

Compound: UO_2
Area sampled: special unit
Sampling time: 1 min

Limit size, μ	No. frequency										ΣN	Average group size, μ	Mass factor, D^3	Weighted groups, $\Sigma N \times D^3$	Mass frequency, %	% less than size limit
	N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8	N_9	N_{10}						
0.5	1	2	8	0	10	16	5	6	7	3	58	0.25	0.0156	0.905	0.0168	0.0168
1.0	53	60	38	43	40	43	46	35	42	40	440	0.75	0.422	185.7	3.44	3.46
1.5	25	23	18	28	21	16	34	28	28	18	239	1.25	1.95	466.1	8.62	12.1
2.0	14	8	17	11	14	11	16	16	22	16	145	1.75	5.35	775.8	14.3	26.4
3.0	4	6	13	14	13	12	8	9	19	14	112	2.50	15.6	1,747	32.3	58.7
4.0	2	1	4	3	1	1	5	3	5	4	29	3.50	42.9	1,244	22.9	81.6
5.0	1	0	2	1	1	1	1	1	2	2	11	4.50	91.1	1,002	18.5	100
6.0												5.50	166			
7.0												6.50	275			
8.0												7.50	422			
9.0												8.50	614			
10.0												9.50	857			

Table 10.14 — Size-Mass Distribution

Instrument:	Modified Cascade Impactor	Compound:	UO_2
Stage:	C	Area sampled:	special unit
Method I:	resin collected	Sampling time:	1 min

Limit size, μ	No. frequency										ΣN	Average group size, μ	Mass factor, D^3	Weighted groups, ΣN × D^3	Mass frequency, %	% less than size limit
	N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8	N_9	N_{10}						
0.5	1	7	1	1	4	1	8	8	7	1	39	0.25	0.0156	0.608	0.0346	0.0346
1.0	62	51	54	52	49	36	56	48	60	59	527	0.75	0.422	222.4	12.7	12.7
1.5	27	30	30	40	37	38	26	25	25	25	303	1.25	1.95	590.9	33.6	46.3
2.0	10	10	12	6	10	22	9	17	7	7	110	1.75	5.35	588.5	33.4	79.7
3.0	0	2	3	1	0	3	1	1	1	8	20	2.50	15.6	312	17.8	97.5
4.0	0	0	0	0	0	0	0	1	0	0	1	3.50	42.9	42.9	2.45	100
5.0												4.50	91.1			
6.0												5.50	166			
7.0												6.50	275			
8.0												7.50	422			
9.0												8.50	614			
10.0												9.50	857			

Table 10.15 — Size-Mass Distribution

Instrument:	Modified Cascade Impactor	Compound:	UO_2
Stage:	D	Area sampled:	special unit
Method I:	resin collected	Sampling time:	1 min

Limit size, μ	No. frequency										ΣN	Average group size, μ	Mass factor, D^3	Weighted groups, ΣN × D^3	Mass frequency, %	% less than size limit
	N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8	N_9	N_{10}						
0.5	9	14	7	5	2	6	31	29	37	42	182	0.25	0.0156	2.839	0.420	0.420
1.0	76	77	77	74	63	52	67	64	60	52	662	0.75	0.422	279.4	41.2	41.6
1.5	14	9	11	18	33	35	2	7	3	6	138	1.25	1.95	269.1	39.7	81.3
2.0	1	0	5	2	2	5	0	0	0	0	15	1.75	5.35	80.25	11.7	93.0
3.0	0	0	0	1	0	2	0	0	0	0	3	2.50	15.6	46.8	6.91	100
4.0											0	3.50	42.9			
5.0												4.50	91.1			
6.0												5.50	166			
7.0												6.50	275			
8.0												7.50	422			
9.0												8.50	614			
10.0												9.50	857			

found as a result of chemical analysis (Table 10.16). The weighted mass frequency in each size class for all stages was then summed up, and the result used to calculate the total size-mass distribution. This procedure is shown for each method in Tables 10.17, 10.18, and 10.19.

Table 10.16—Average Chemical Analytical Values on Resin- -and Dry-collected Samples of UO_2 Dust

	Resin-collected		Dry-collected	
Stage	Average mass, %	Cumulative percentage	Average mass, %	Cumulative percentage
A	23.2	88.4	5.38	96.0
B	46.1	53.7	27.1	79.3
C	16.4	22.5	18.5	57.0
D	8.7	10.0	8.8	43.3
E	5.6	2.8	38.9	19.5

The distributions of individual stages and the total distributions for each method are given on a log-probability plot in Figs. 10.22, 10.23, and 10.24. The effective stage constants (listed in Table 10.20) were determined from the intercepts on the total distribution curves of the mass values determined by chemical analysis. The stage constants determined as the medians of the distributions derived from measurements on individual stages are also shown. The close correspondence of the two sets of constants indicates agreement in the results of both procedures.

Because none of the curves for total distribution by the three methods was normal (as would be represented by straight lines), the best-fitting curve was used. From these curves, corrections were made to determine the final calibration curve for the Modified Cascade Impactor for the purpose of representing the condition of resin collection and measurement on a selenium mount. These corrections were determined graphically on the log-probability plot illustrated in Fig. 10.25. The improvements in measurement as represented by selenium mounting (method III) were applied point by point along the percentage axis to method II. This resultant was then corrected for improvement in collecting efficiency as represented by resin coating (method I) in the same manner. The final calibration curve was drawn through the corrected points. The graphic procedure was used in order to avoid a complex mathematical treatment. This final calibration curve represents a normal probability distribution (straight line)

Table 10.17 — Total Size-Mass Distribution, Summation of All Stages of the Modified Cascade Impactor

Method I: Resin Collected, Resin Mounted

Stage	A		B		C		D		E		Total weighted frequency	Summation less than upper limit	% less than upper limit
Mass factor, %	23.2		46.1		16.4		8.7		5.6				
Limit size, μ	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency			
0.5	0.0101	0.234	0.0168	0.774	0.0346	0.568	0.420	3.65	6.00	33.6	38.8	38.8	0.39
1.0	0.615	14.3	3.44	159	12.7	208	41.2	358	76.0	425.6	1,165	1,204	12.0
1.5	0.924	21.5	8.62	397	33.6	552	39.7	345.4	15.0	84.0	1,400	2,604	26.0
2.0	1.35	31.3	14.3	659	33.4	548	11.7	101.8	3.00	16.8	1,357	3,961	39.6
3.0	3.70	85.9	32.3	1490	17.8	292	6.91	60.1			1,928	5,889	58.8
4.0	8.96	208	22.9	1060	2.45	40.2					1,308	7,197	71.9
5.0	15.4	357	18.5	853							1,210	8,408	84.0
6.0	22.1	513									513	8,920	89.1
7.0	23.7	550									550	9,470	94.6
8.0	13.6	316									316	9,786	97.8
9.0	6.62	154									154	9,940	99.3
10.0	3.08	71.5									71.5	10,011	100

Table 10.18—Total Size-Mass Distribution, Summation of All Stages of the Modified Cascade Impactor

Method II: Dry Collected, Resin Mounted

Stage	A		B		C		D		E		Total weighted frequency	Summation less than upper limit	% less than upper limit
Mass factor, %	5.38		27.1		18.5		8.8		38.9				
Limit size, μ	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency			
0.5	0.0426	0.228	0.317	8.58	0.677	12.5	1.06	9.33	1.7	66.1	96.7	96.7	0.97
1.0	2.24	12.1	11.4	309	20.5	379	28.9	254	41.3	1,607	2,561	2,658	27.0
1.5	4.39	23.6	17.5	474	33.5	620	33.8	297	40.0	1,556	2,971	5,629	57.0
2.0	4.96	26.7	23.6	640	29.4	544	24.5	216	13.0	506	1,933	7,562	76.5
3.0	12.5	67.3	28.4	770	16.0	296	11.7	103	4.0	156	1,392	8,954	90.6
4.0	15.0	80.7	13.5	366							447	9,401	95.2
5.0	18.3	98.5	5.72	155							254	9,655	97.8
6.0	16.7	89.8									89.8	9,745	98.7
7.0	20.7	111									111	9,856	99.8
8.0	5.26	28.5									28.5	9,884	100
9.0													
10.0													

Table 10.19—Total Size-Mass Distribution, Summation of All Stages of the Modified Cascade Impactor

Method III: Dry Collected, Selenium Mounted

Stage	A		B		C		D		E		Total weighted frequency	Summation less than upper limit	% less than upper limit
Mass factor, %	5.38		27.1		18.5		8.8		38.9				
Limit size, μ	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency	Mass frequency, %	Weighted frequency			
0.5	0.0632	0.339	0.440	11.9	4.19	77.4	6.82	60.0	25.0	972.5	1.122	1,122	11.36
1.0	1.36	7.32	18.8	509	64.1	1,190	69.4	610.7	71.5	2,781.4	5,098	6,220	63.1
1.5	3.30	17.8	32.4	878	27.2	503	205	180.4	3.5	136.2	1.715	7,935	80.5
2.0	3.93	21.1	29.2	795	4.65	86.0	3.32	29.2			931.3	8,866	89.9
3.0	10.2	54.9	14.8	401							455.9	9,322	94.5
4.0	27.6	148	4.04	109							257	9,579	97.0
5.0	25.7	138									138	9,717	98.5
6.0	18.7	101									101	9,818	99.5
7.0	9.30	50.0									50	9,868	100
8.0													
9.0													
10.0													

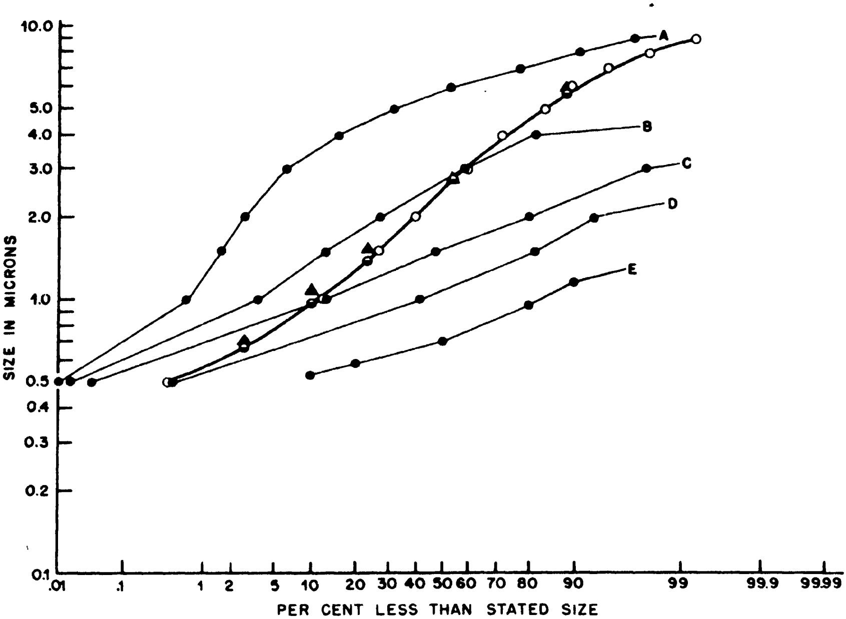


Fig. 10.22—Calibration curves of the Modified Cascade Impactor, showing size-mass distribution of a standard UO_2 atmosphere, as determined by method I. The distributions obtained by measurement of the sizes on each of the corresponding stages are shown by curves A, B, C, and D. The most probable distribution for sizes on stage E is shown by curve E. Solid dots represent actual data. The total size-mass distribution obtained by method I is plotted by circles. ◒ represents stage-size calibration values determined from the total distribution curve as effective size constants. ▲ represents results obtained by plotting stage-distribution medians as stage-size constants.

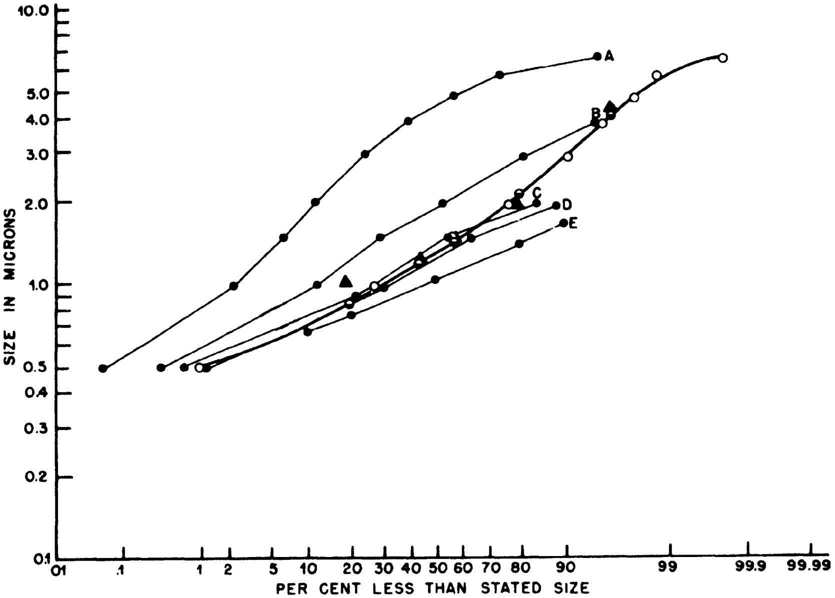


Fig. 10.23—Calibration curves of the Modified Cascade Impactor, showing size-mass distribution of a standard UO_2 atmosphere, as determined by method II. The distributions obtained by measurement of the sizes on each of the corresponding stages are shown by curves A, B, C, and D. The most probable distribution for sizes on stage E is shown by curve E. Solid dots represent actual data. The total size-mass distribution obtained by method I is plotted by circles. ◒ represents stage-size calibration values determined from the total distribution curve as effective size constants. ▲ represents results obtained by plotting stage-distribution medians as stage-size constants.

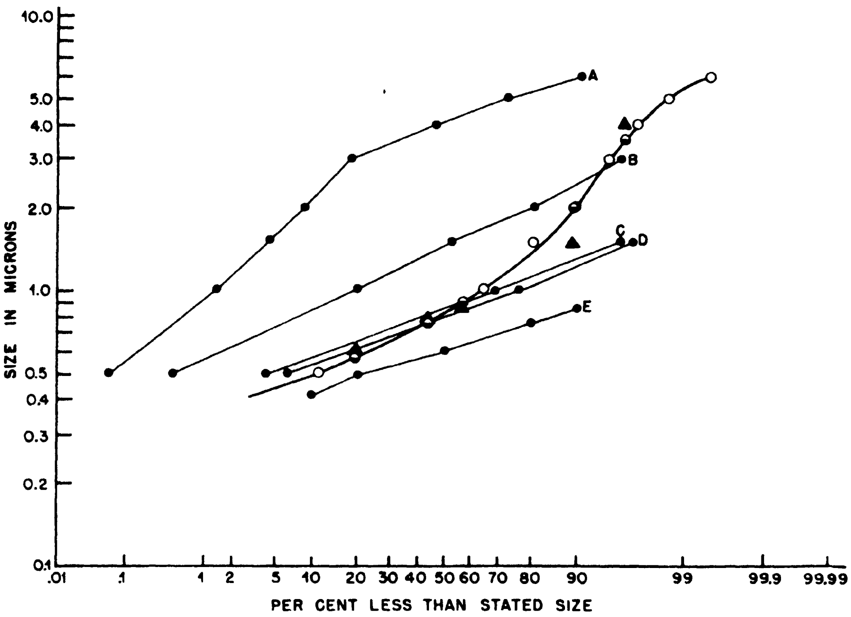


Fig. 10.24—Calibration curves of the Modified Cascade Impactor, showing size-mass distribution of a standard UO_2 atmosphere, as determined by method III. The distributions obtained by measurement of the sizes on each of the corresponding stages are shown by curves A, B, C, and D. The most probable distribution for sizes on stage E is shown by curve E. Solid dots represent actual data. The total size-mass distribution obtained by method I is plotted by circles. ⊖ represents stage-size calibration values determined from the total distribution curve as effective size constants. ▲ represents results obtained by plotting stage-distribution medians as stage-size constants.

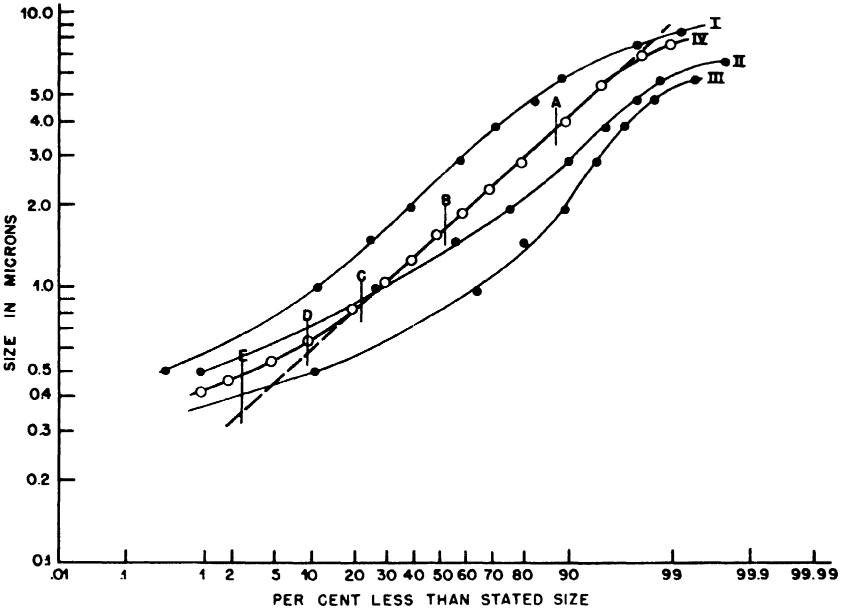


Fig. 10.25 — Final calibration curve of the Modified Cascade Impactor, showing size-mass distribution of a standard UO_2 atmosphere. Curve I, total size-mass distribution obtained by method I; curve II, total size-mass distribution obtained by method II; curve III, total size-mass distribution obtained by method III; curve IV, corrected distribution curve.

Table 10.20—Mass Constants of the Stages Determined by Methods I, II, and III

	Method I		Method II		Method III	
Stage	Effective size constant, μ	Stage median, μ	Effective size constant, μ	Stage median, μ	Effective size constant, μ	Stage median, μ
A	5.7	6.0	4.25	4.6	3.55	4.00
B	2.72	2.72	2.19	2.0	1.95	1.45
C	1.40	1.53	1.45	1.45	0.85	0.90
D	0.95	1.08	1.20	1.21	0.75	0.79
E	0.67	0.71	0.85	1.02	0.56	0.61

except at the extremes. It is a convenient and accepted practice to use the best straight line on the log-probability plot to represent a size distribution. Therefore, the extremes were neglected, and the straight part of the curve extrapolated. It is obvious, however, that the size-mass distributions determined by such procedures apply only to the straight part of the curve and not to the extremes.

The final calibration constants were determined as the effective size intercepts on the final calibration curve as indicated by points A, B, C, D, and E on Fig. 10.25. These values are 3.95, 1.66, 0.87, 0.58, and 0.35 μ, respectively, for UO_2.

(5) The Application of UO_2 Constants to Other Compounds. The impaction efficiency of a jet can be defined as the percentage ratio of the number of particles hitting the collecting stage to the number of particles entering the jet, assuming an ideal condition under which all particles hitting the stage are retained. These efficiency values can be obtained directly from the measured results in terms of the percentage frequency as count or mass found for a size class of a specific jet.

Under conditions of equal impaction efficiencies, May[37] shows that the following relationship can be derived for the British Cascade Impactor stages

$$\rho V D_n^2 / \eta L = I_n$$

where ρ = the density of the particle (assumed large with respect to air).
V = the velocity of the jet across the slit.
η = the viscosity of air.
L = a characteristic length of the system taken as the slit width.
D_n = the diameter of the particle (if spherical) at the impaction efficiency n.
I_n = a constant at the impaction efficiency n.

May found that for materials of unit density the values of I_n for a given n were relatively constant, indicating that the British Cascade Impactor was an efficient instrument for sampling air-borne material. Applying the foregoing formula to his results it is possible to check the values obtained with UO_2 and also to calculate the impactor constants for materials of any stated density.

By use of May's original data, a calculation was made of the expected count distributions for stages A, B, C, and D for a material of the density of UO_2, 10.9, and for a sampling rate of 14.0 liters/min. The calculated count distributions were then converted to mass distributions, and the mass medians of each determined. The values obtained were 4.76, 1.27, 0.64, and 0.38 μ for stages A, B, C, and D, respectively. Considering the fact that stage A of the Modified Cascade Impactor is different in design and that the distribution on this stage is a variable dependent on the upper limits of the sampled atmospheres, the experimentally determined value of calibration constant of 3.95 μ shows good agreement. Stages B, C, and D of the Modified Cascade Impactor have the same slit dimensions and internal design as those of the British instrument. The experimental values for these stages were 1.66, 0.86, and 0.58 μ, which were in excellent agreement considering the fact that May's results were based on liquid droplets of spherical shape, compared to irregular solid particles. Thus the relationship for equal impaction efficiencies may be considered valid for the modified impactor.

The equal-impaction-efficiency formula can be rewritten in terms of size and density. In this form

$$\rho D_n^2 = \frac{I_n L \eta}{V} = \text{constant}$$

it can be used to determine impactor constants for a material of any density at a fixed sampling velocity. Using the experimentally determined effective size constants of UO_2 as standard, values for certain other uranium compounds were calculated. These are presented in Table 10.21. The generalized relationship of density to the constants for the modified impactor are shown in Fig. 10.26. From the curves the constants for any material of known density can be found.

(6) Particle-size Distribution of Uranium Dusts. A series of samples was taken with the Modified Cascade Impactor to determine the size-mass distributions of uranium-dust atmospheres, making use of the previously determined impactor constants. Studies were made of four uranium compounds, uranyl nitrate, $UO_2(NO_3)_2$, uranium tetrachloride, UCl_4, uranium tetrafluoride, UF_4, and uranium dioxide, UO_2, at the 10 different concentration levels used in inhalation studies.

Table 10.21—Stage-calibration Values of the Modified Cascade Impactor for the Uranium Compounds Used in the Dust-inhalation Studies

Compound	Density	Stage calibration values, μ				
		A	B	C	D	E
UO_2	10.9	3.95	1.66	0.87	0.58	0.35
UF_4	7.1	4.89	2.05	1.07	0.72	0.43
UCl_4	4.86	5.92	2.48	1.30	0.87	0.53
$UO_2(NO_3)_2.6H_2O$	2.81	7.79	3.27	1.71	1.14	0.69

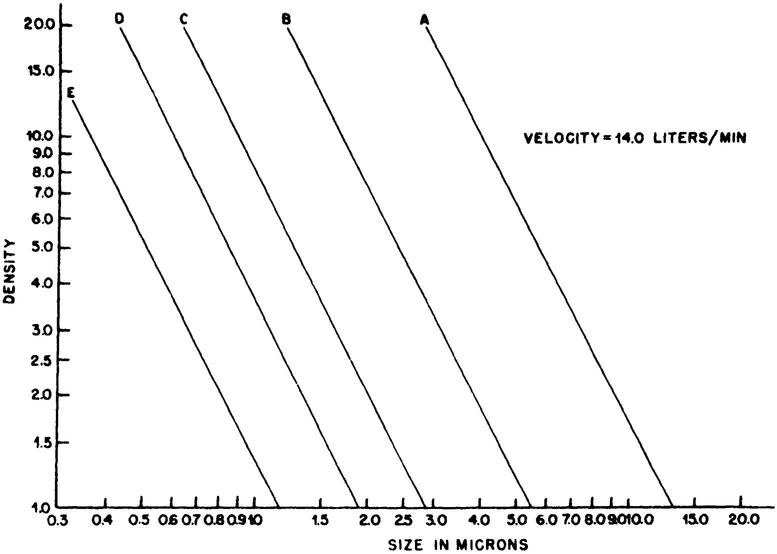


Fig. 10.26—Calculated stage-calibration values of the Modified Cascade Impactor for compounds of different densities.

The distributions for each determination were expressed in terms of the median size and the standard deviation. Information is also given for the chamber-dust concentration, air temperature, and relative humidity at the time of sampling, although at the present time, with the limited quantity of data available, no correlations of these factors with size distributions can yet be made.

i. Uranium Dioxide, UO_2. The results obtained on UO_2 for both the 1- and the 10-mg levels are shown in Table 10.22. For the 10-mg level the average value for the median mass size was 1.23 μ, with an

Table 10.22—Size-Mass Distributions of Uranium Dioxide Occurring in the Animal-exposure Studies*

Concentration level of compound, mg U/cu m	Sample No.	Chamber concentration, mg U/cu m†	Temp., °F†	Humidity, %†	Median size, μ	Standard deviation
1.0	1	1.13	78	46	1.40	2.14
	2	1.19	72	45	1.15	2.42
	3	0.99	71	48	1.10	2.64
	4	1.10	70	48	1.29	2.36
	5	1.10	74	54	1.32	2.14
	6	1.13	77	52	1.24	2.33
10.0	1	9.67	79	40	1.09	2.00
	2	9.62	77	45	1.43	1.96
	3	10.12	73	46	1.28	2.62
	4	7.73	75	47	1.07	2.34
	5	9.79	79	57	1.31	2.79

* Results obtained with the Modified Cascade Impactor.

† Values obtained at the time of sampling.

Table 10.23—Size-Mass Distributions of Uranium Tetrafluoride Occurring in the Animal-exposure Studies*

Concentration level of compound, mg U/cu m	Sample No.	Chamber concentration, mg U/cu m†	Temp., °F†	Humidity, %†	Median size, μ	Standard deviation
0.5	1	0.67	75	74	2.13	2.96
	2	0.39	71	45	1.23	2.36
	3	0.34	72	45	1.26	2.86
	4	0.15	73	46	1.20	2.66
	5	0.45	74	54	1.02	2.99
	6	0.30	74	47	1.92	1.73
3.0	1	2.9	79	43	1.12	2.10
	2	3.8	78	33	1.02	2.38
	3	2.9	73	42	1.32	2.19
	4	2.0	76	62	1.16	2.89
	5	3.4	78	53	1.66	2.24
	6	2.6	79	40	1.82	2.88

* Results obtained with the Modified Cascade Impactor.

† Values obtained at time of sampling.

average standard deviation value of 2.34. The median mass sizes showed a range of from 1.07 to 1.43 μ. The standard deviations ranged from 1.96 to 2.79.

The 1-mg level showed approximately the same value for the average median mass size of 1.25 with a range from 1.10 to 1.40 μ. The average standard deviation was almost the same as that for the high levels, 2.33. The range, however, was slightly smaller with values of from 2.14 to 2.64.

ii. Uranium Tetrafluoride. The results obtained on uranium tetrafluoride for both the 0.5-mg and the 3-mg levels are shown in Table 10.23. For the 0.5-mg level, the average value for the mass-size median was 1.46 μ, with a range of values obtained between 1.02 and 2.13 μ. The average standard deviation was 2.59, with a range of 1.73 to 2.99.

For the 3-mg level the average value was 1.35, with a range of 1.02 to 1.82. The average standard deviation was 2.44, with a range of 2.10 to 2.89.

iii. Uranyl Nitrate. The results obtained on uranyl nitrate for the four concentration-level studies are shown in Tables 10.24 and 10.25. The average values for the mass-size medians and the standard deviations for each of the four levels are given in the following tabulation.

Average Size-Mass Distributions of Uranyl Nitrate Dust at Four Concentrations

Concentration level of compound, mg U/cu m	Average median size, μ	Standard deviation
0.05	2.75	2.46
0.15	2.93	2.36
0.45	4.04	2.69
1-1.5	3.12	2.34

Variations from the average median sizes were large, as indicated for the 0.05-mg level, with a range from 1.58 to 4.40 μ. Standard deviations showed little variation, with values ranging from 2.22 to 3.01 for the same level. A comparison of size values with concentration levels suggests that the increase in concentration is accompanied by increase in particle size in the nitrate units.

iv. Uranium Tetrachloride. The results on uranium chloride at the 0.05- and 0.2-mg levels are shown in Table 10.26. The values obtained for both levels were 1.60 μ for the 0.05-mg level and 1.73 μ

Table 10.24—Size-Mass Distributions of Uranyl Nitrate Occurring in the Animal-exposure Studies*

Concentration level of compound, mg U/cu m	Sample No.	Chamber concentration, mg U/cu m†	Temp., °F†	Humidity, %†	Median size, μ	Standard deviation
0.05	1	0.033	75	37	2.60	2.22
	2	0.033	74	39	2.66	2.25
	3	0.047	75	44	3.20	2.44
	4	0.050	73	50	1.58	3.01
	5	0.037	75	58	2.62	2.33
	6	0.030	74	50	4.40	2.50
	7	0.047	76	44	2.22	2.52
0.15	1	0.31	76	41	2.55	2.35
	2	0.30	79	53	2.85	2.53
	3	0.44	74	50	2.32	2.24
	4	0.14	73	57	2.71	2.32
	5	0.17	74	69	3.09	2.60
	6	0.14	75	62	4.05	2.34
	7	0.12	74	61	3.00	2.20

*Results obtained with the Modified Cascade Impactor.

†Values obtained at time of sampling.

Table 10.25—Size-Mass Distributions of Uranyl Nitrate Occurring in the Animal-exposure Studies*

Concentration level of compound, mg U/cu m	Sample No.	Chamber concentration, mg U/cu m†	Temp., °F†	Humidity, %†	Median size, μ	Standard deviation
0.45	1	0.50	77	35	3.87	2.27
	2	0.52	79	43	3.60	2.62
	3	0.46	81	41	4.75	2.32
	4	0.37	76	44	3.95	3 56
1.0-1.5	1	0.20	75	62	2.75	2.44
	2	1.68	80	47	2.29	2.18
	3	3.02	75	40	4.0	2.22
	4	3.10	73	53	3.75	2.48
	5	1.71	75	54	3.62	2.51
	6	3.20	75	54	2.22	2.30
	7	2.28	75	47	3.22	2.31

*Results obtained with the Modified Cascade Impactor.

†Values obtained at time of sampling.

for the 0.2-mg U/cu m. Standard deviations were 2.38 and 2.04, respectively. The range of sizes for the 0.05-mg level were 1.29 to 1.95 μ. For the 0.2-mg level the range was found to extend from 1.20 to 2.07 μ.

Table 10.26—Size-Mass Distributions of Uranium Tetrachloride Occurring in the Animal-exposure Studies*

Concentration level of compound, mg U/cu m	Sample No.	Chamber concentration, mg U/cu m†	Temp., °F†	Humidity, %†	Median size, μ	Standard deviation
0.05	1	0.05	73	50	1.84	2.17
	2	0.05	69	67	1.64	2.35
	3	0.02	74	47	1.62	2.39
	4	0.02	76	44	1.29	2.19
	5	0.02	71	56	1.52	2.56
	6	0.04	76	55	1.58	2.21
	7	0.02	78	56	1.95	2.82
0.2	1	0.12	72	49	1.72	1.89
	2	0.23	74	54	1.51	2.17
	3	0.21	78	43	1.20	2.41
	4	0.22	76	38	1.89	2.04
	5	0.15	82	64	2.02	1.68
	6	0.31	79	43	1.73	2.11
	7	0.24	81	43	2.07	2.01

*Results obtained with the Modified Cascade Impactor.

†Values obtained at time of sampling.

(e) Particle-size Distribution of UO_2F_2 Fume. The UO_2F_2 fume produced as a result of the hydrolysis of UF_6 by the atmosphere was found to be a true fume in that all the sizes measured were well below 1 μ. Preliminary study of samples of this fume with an optical microscope showed that the major portion of the size distribution was at or below the resolution limits and that the electron microscope was the only suitable measuring instrument. The thermal precipitator[20] was found to be the most practical instrument for routine sampling. This instrument was modified to permit direct collection of the particulate material on prepared electron microscope grids.[42] The collected samples were photographed in an electron microscope (developed and constructed by F. Bishop of the University of Rochester Medical School) and sizes were measured from these photographs by projection on a calibrated grid. A total magnification of 20,000× was used; Fig. 10.27 illustrates a typical electron micrograph obtained.

The particle-size distributions obtained on a series of six samples at the 0.2-mg level of the UO_2F_2 fume are given in Table 10.27 in terms of both the count and the mass-distribution parameters. For the size-count distributions the average value of the median sizes was found to be 0.056 μ with an average standard-deviation value of 1.66. The median sizes ranged from 0.048 to 0.064 μ, with a corresponding range in standard deviation from 1.95 to 1.54.

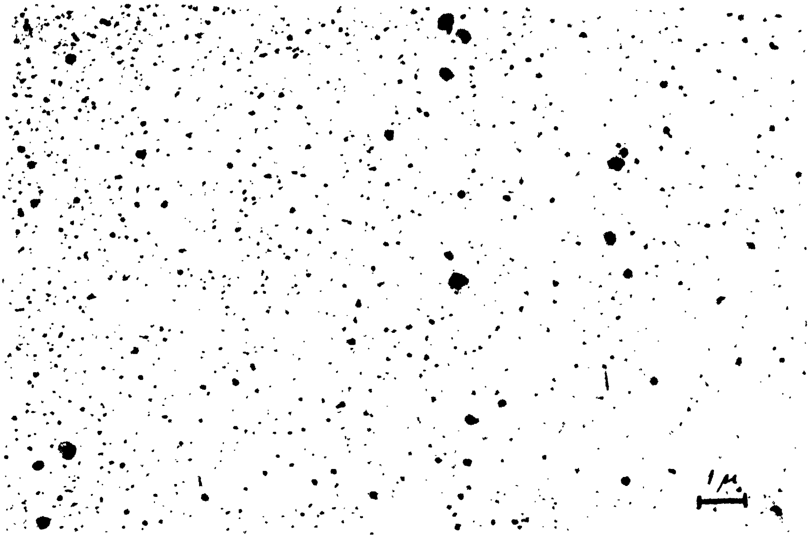


Fig. 10.27—Electron micrograph of a UO_2F_2 fume resulting from the introduction of UF_6 into the atmosphere.

For the size-mass distributions the average value of the median sizes was found to be 0.102 μ with an average standard-deviation value of 1.42. The median sizes ranged from 0.086 to 0.128 μ. Standard-deviation values ranged from 1.31 to 1.60.

8.3 The Relation of Particle Size to Toxicity. (Report and work by Charles W. LaBelle.) Authors who have discussed the dust problem have been aware of the possibility that the particle size of the inhaled dust is one of the determinative factors in the retention of the material within the animal body. Thus Findeisen[43] on theoretical grounds has calculated the probable percentage retention of particles of various sizes within the different regions of the respiratory system, and several authors have attempted with greater or lesser success to measure this differential response by direct experiment.

Table 10.27 — Particle-size Distributions of UO_2F_2 Fume (Electron Microscope)

Sample No.	Count distribution Median size, μ	Count distribution σ_g	Mass distribution Median size, μ	Mass distribution σ_g
1	0.048	1.95	0.106	1.39
2	0.053	1.60	0.096	1.37
3	0.054	1.59	0.086	1.31
4	0.064	1.54	0.096	1.60
5	0.055	1.63	0.098	1.37
6	0.062	1.67	0.128	1.48

Little attention has been paid, however, to the possibility that one size of particle may be inherently more toxic than another size. The experiment to be described represents an attempt to isolate this factor by studying the toxicological reaction of rats under conditions in which the size of the particles introduced into the lungs is the only variable.

In any toxicological experiment involving the inhalation of material in suspension in air, the difficulties of determining actual dosage in the customary units of mass of toxic agent per unit body weight are sufficiently great to preclude the possibility of following small shifts in toxicity in response to changes in a given condition of exposure, unless a group of animals sufficiently numerous to be statistically significant is used for each determination. The injection of saline suspensions of toxic agents into the tracheae of anesthetized animals has been found to permit fairly accurate dosage of insoluble materials resulting in a sharp reduction in both the number of animals and the time required to produce differences in response that are statistically valid. This method of administration has been employed to study the variation in toxicity resulting from a progressive decrease in the mean particle size of uranium dioxide powders.

(a) History of the Method. The use of intratracheal injection to study the physiological reaction of the lung to foreign materials has been credited by one investigator to an obscure report by Tanqueral des Planches, as early as 1839. The first extended experiments using this technique were reported by Slavjansky[44] in 1869 in one of the earliest studies on pneumoconiosis and by Tchistovitch[45] in 1889 in one of the first studies on the phagocytic reactions of the lung.

The technique became very popular in the early twenties. A number of studies of the origin of the lung phagocytes were made by Sewell,[46] Foot,[47] McJunkin,[48] and Permar,[49] all of whom used intratracheal injections to stimulate the production of free phagocytes within the alveoli. The method was applied by Lehmann[50] to the study of the toxicology of metal-fume fever, reapplied by Winternitz and his associates[51] to war-gas pathology, and employed brilliantly by Minot[52] and his associate Blumgart[53] in 1923 to study the physiology of lead poisoning. The method was used by Axelsson and Bringel[54] to study the development of tuberculosis in pneumoconiosis and finally reinvented by Kettle and Hilton in 1932[55] for the same purpose.

(b) Experimental Techniques. (1) Preparation of Particle-size Fractions. A sample of uranium dioxide was ground in a micropulverizer until substantially all the particles were below 10 μ in size. This powder was suspended in water and separated into seven fractions by a modification of the method of Cummings.[56] Briefly the method consists in allowing the suspension to settle for an initial period t_1, decanting from the sediment, resettling for a longer period t_2, and repeating the process as many times as desired, in this case up to t_7. Each fraction is then recycled after the manner of a fractional crystallization, until the overlapping between fractions is reduced to any desired value. The fractions employed in the present work were recycled 21 times, following the flow diagram (Fig. 10.28), producing cuts in which successive fractions did not overlap by more than 10 per cent.

(2) The Technique of Intratracheal Injection. Each fraction was diluted with water to a solids content of 20 mg/ml, and sodium chloride was added to a concentration of 0.89 per cent. Albino rats were anesthetized with ether and arranged on an operating board. The ventral surface of the neck was shaved and scrubbed lightly with alcohol, and a ½-in. incision made on the mid-line directly above the thyroid, cutting through both skin and subcutaneous tissue until the sternohyoid muscle was reached. A pair of curved forceps was used to spread the two halves of the muscle exposing the trachea. The wound was then covered with a saline sponge while a tuberculin syringe was filled with the proper amount of uranium oxide suspension (1.5 ml/kg, or 30 mg of solids per kilogram). The rat was tilted head up to about 45 deg, the trachea revisualized, and the charge in the syringe injected into the trachea, rotating the barrel of the syringe during the injection and forcing the suspension into the trachea in small spurts to coincide with the animal's inhalations. A reasonably

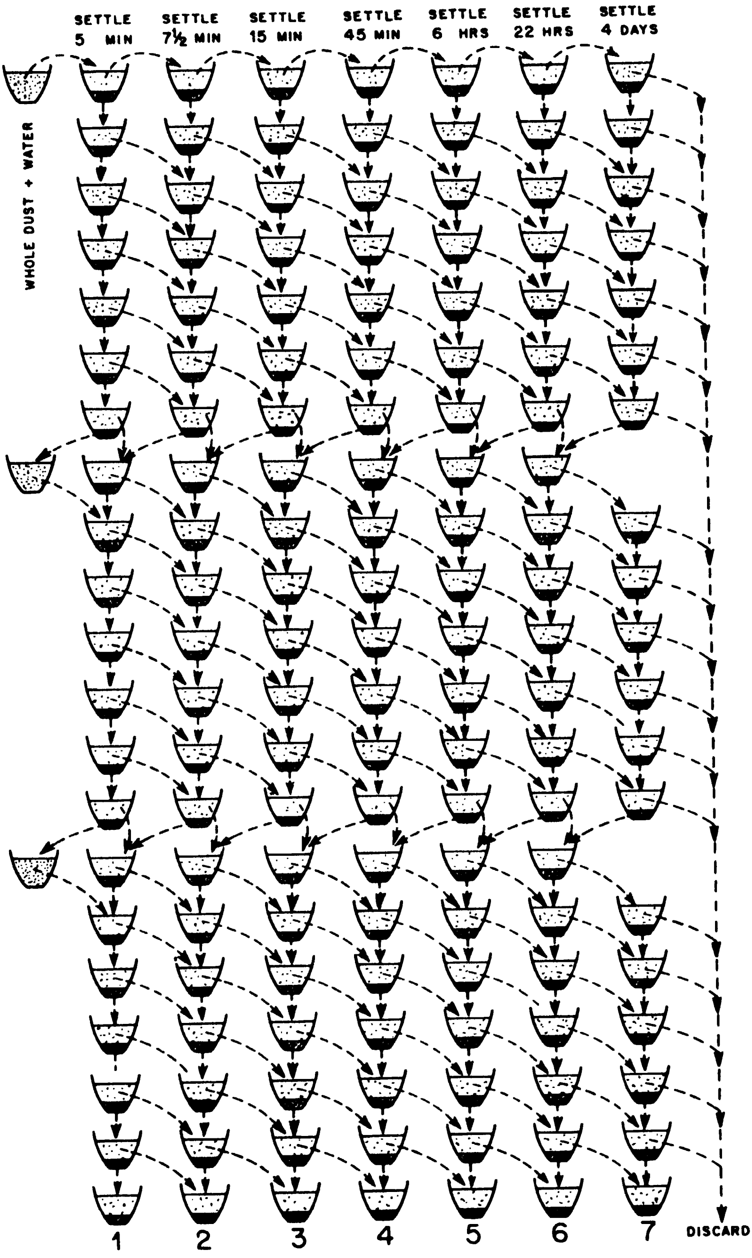


Fig. 10.28 — Flow sheet for fractionation of dust.

quantitative transfer is thus possible. A single loop suture through the subcutaneous tissues and two or three mattress sutures in the skin wound completed the operation.

(c) Toxicologic Procedures. Six rats were used for each fraction of uranium dioxide. The weights of the animals were determined twice daily for the first 2 weeks and daily thereafter. At the end of 1, 2, and 4 weeks from the date of the operation, one animal was sacrificed from each group. In every case the animals selected were those whose weight changes most nearly approximated the mean weight change of the group. This was done so as to secure data that would best represent the entire group.

Each of these animals was sacrificed according to a procedure that permitted the determination of the following variables: (1) nonprotein nitrogen of the blood, (2) kidney function as measured by the ability of the animal to excrete PSP, (3) histological changes in the lung and kidney, and (4) the uranium content of the lung, femur, and kidney. The routine procedure employed in the terminal sacrifice of the animals studied consisted of the following described steps.

The animal was anesthetized with nembutal, and 1.0 ml of PSP solution was injected into the carotid artery. Exactly 30 min later the urinary bladder was exposed, clamped off completely with hemostats, excised from the body, and placed in 20 ml of distilled water. A sample of blood was withdrawn from the heart, and the lung, kidneys, and one femur were removed from the body. A small portion of lung and of one kidney were placed in Bouin's fluid for histological study; the remainder of the lung, the opposite kidney, and the femur were preserved separately in alcohol pending analysis for uranium. The excised bladder was cut open while under the surface of the water, the mixture was filtered, and an additional 5 ml of water used to wash it. The filtrate was made alkaline with 1 drop of 10 per cent sodium hydroxide and filtered a second time, and the PSP content of the filtrate determined in a photoelectric colorimeter. The nonprotein-nitrogen content of the blood was determined by the routine blood-chemistry laboratory; the uranium content of the liver, kidney, and femur was determined by the routine-tissue-analysis laboratory through the fluorophotometric method (Chap. 2).

(d) Results and Discussion. In general a maximal toxicologic response was found 1 week after the injection of UO_2 dust, with a return thereafter toward more normal values. The 1-week data were therefore found to be of greatest value in differentiating the effect of the various-size fractions. Results given in Fig. 10.29 and Table 10.28 indicate that the toxic effect of UO_2 dust varied inversely with the particle size.

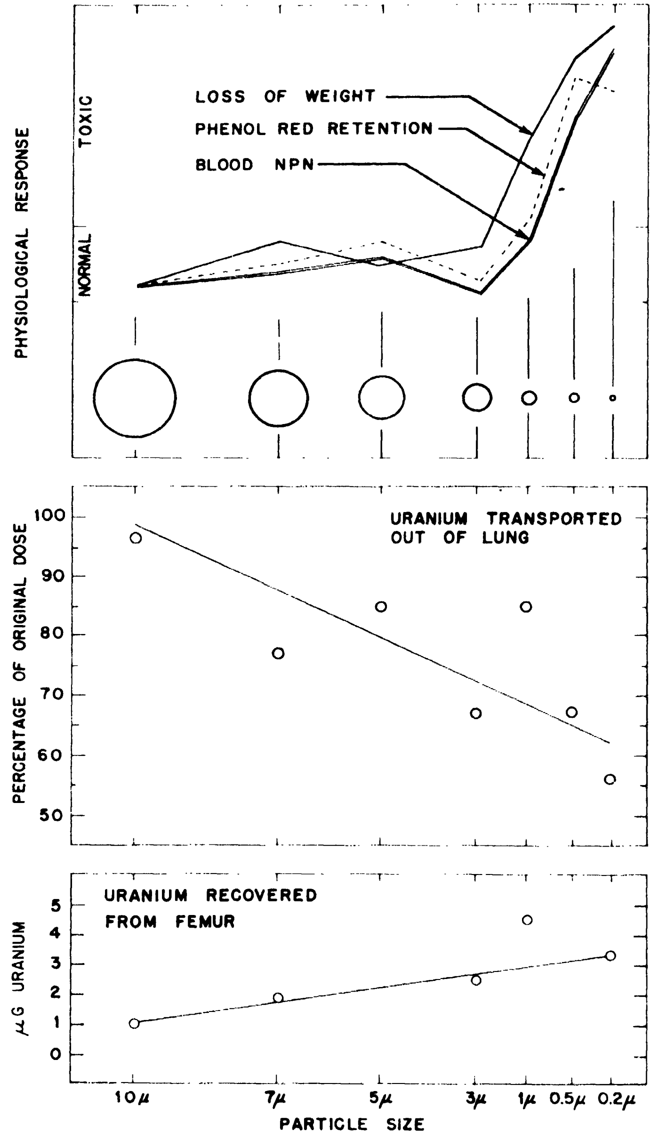


Fig. 10.29 — Changes in toxicologic responses and body transport of uranium in rats following intratracheal injection of 30 mg UO_2/kg of body weight in graded particle sizes. Upper, retention of phenolsulfonphthalein dye (phenol red), body-weight changes, and changes in nonprotein nitrogen of blood; middle, transportation of uranium from lung; lower, deposition of uranium in femur.

With the size fraction 7 (mean size 0.2 μ) there was a loss of body weight of over 10 per cent, a rise in blood nonprotein nitrogen to 120 mg % and decrease in PSP excretion to less than 1 per cent. Toxic effects were also observed with size fractions 5 and 6 (mean sizes of 0.6 and 1.0 μ) but not with any of the larger sizes. The magnitude of the variations was significant, since the range involved between the coarse and fine fractions is roughly that which would be produced by a 500 to 1000 per cent change in dosage of any one fraction.

Table 10.28 — Effect of Particle Size on Toxicity of Uranium Dioxide

	Fraction						
Variable	1	2	3	4	5	6	7
Mean diameter of particle, μ	10	7	5	3	1	0.6	0.2
Mean body-weight change, %	0	−0.5	−1.2	−1.4	−1.8	−7.0	−10.1
Phenolsulfonphthalein retained, %	45	54	49	52	75	92.5	99.5
Blood nonprotein nitrogen, mg %	40	46	52	36	58	130	120
Uranium:							
Transferred from lung, %*	96	77	85	67	85	67	56
Found in femur, μg	3.1	5.4	4.2	9.9	5.9	13.4	
Found in kidney, μg	0.8	2.0	2.3	3.7	0.9	6.2	4.4

*Percentage of dose administered.

The loss of body weight, although a nonspecific symptom, was a valuable index because measurements could be made daily without any injury to the experimental animals. Determination of blood NPN and PSP excretion required the sacrifice of an animal but were valuable tests because they indicated that a main site of damage was the kidney. This was also borne out by the demonstration of fairly high quantities of uranium in the femur and kidney, definitely establishing uranium as the active causal agent of the observed damage as opposed to the possibility that the mechanical or chemical stimulation of the lung tissue might cause the production of a "toxin" that would in turn damage the kidneys. The uranium content of the femur tended to be greatest in the same animals that evidenced the greatest damage; the uranium content of the kidney, however, did not show this increase to the same extent, suggesting that the uranium was passing through, rather than accumulating, in the kidney tissue. Analyses made at 2 and 4 weeks after uranium injection support this view in showing that bone uranium is cumulative, whereas kidney uranium is not.

Analysis of lungs for uranium content showed an apparent anomaly. In the case of the smaller size fractions, smaller amounts of uranium were transported out of the lung, although larger amounts reached the bones and kidneys. This effect was just the opposite to what would be expected if transport into the general circulation were the main route by which the lungs are cleared of uranium oxide particles. The implication seems to be that either an alternative route is available that does not involve the general circulation at all or that some mechanism within the general circulation, other than excretion by the kidneys, can selectively eliminate large particles of uranium oxide without damage to the organism. It seems probable that the larger sizes are removed from the lung by way of the trachea and intestinal tract, but at the present time this is pure speculation.

(e) Conclusion. It may be concluded that in toxicologic studies involving insoluble dusts, the size of the particles is a factor of great importance. Particles of UO_2 smaller than 1 μ are inherently more dangerous than particles above this limit, a difference that is associated with the movement of the material within the body after its introduction into the lung. The toxicity of an inhaled dust depends on the relative amounts of each size of particle retained in the lung and the specific toxicity of these sizes to the animal.

8.4 Toxicologic Procedures. The usual course followed in setting up the exposure studies was to procure, select, and prepare the experimental animals while the testing of dust feeds was in progress.

(a) Selection of Animal Species. Seven animal species in all were used for the inhalation program, but rarely were all seven used in any one study. More commonly from four to six species were used in the short-term studies; four species only, the dog, rat, rabbit, and guinea pig, were used uniformly on the long-term studies with emphasis being placed on the dog and rat. Selection of the latter was based on species susceptibility. As regards uranium poisoning by inhalation, the dog represents a moderately susceptible species and the rat a relatively resistant species.*

The variety of animals used and their weight range at the time of procurement are shown in Table 10.29.

Beagle dogs were obtained from local kennels until the supply was exhausted. This occurred shortly after the mid-point of the short-term exposure studies. Dogs subsequently obtained were the short-haired mongrel variety and were procured from kennels as far distant as Pennsylvania. Rats and hamsters were obtained solely from our own breeding stock (maintained in a separate building under the direction of Elliott Maynard). Rabbits were supplied from local breeders; guinea pigs were obtained from local breeders and from breeders in Pennsylvania. Mice were obtained from Rockland Farms of New City, N.Y. Cats were obtained from local farmers.

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*The histologic response of the dog to low uranium concentration is equivalent to that of the most susceptible species, the rabbit or cat, but the dog has in addition the advantage of a better capacity for survival and is more suited for numerous experimental procedures than is either the cat or rabbit. The chief advantage of the rat for uranium studies lies in its regenerative capacity following renal injury and in the ease of use of large numbers.

Table 10.29 — Variety and Weight Range of Animal Species

Animal species	Variety	Weight range
Dog	Beagle; short-haired mongrel	6 – 11 kg
Cat	Short-haired domestic	1.5 – 3 kg
Rabbit	Albino; Belgian hare; Chinchilla; New Zealand	2 – 4 kg
Guinea pig	Mixed English	300 – 900 g
Rat	Wistar albino	50 – 250 g
Mouse	Swiss albino	18 – 25 g
Hamster	Golden (Cricetus auratus)	75 – 100 g

(b) Number of Animals. Approximately 5 dogs, 20 rabbits, 30 rats, 30 guinea pigs, and 50 mice were used to test the toxicity at each level in the 30-day studies. This number was by no means uniform. In certain instances in which the purposes of the experiment would be better suited, an increase of certain species was used, and certain others were omitted. For the 1-year studies, 20 dogs and 150 to 200 rats were used for each level tested. In addition, 20 rabbits and 30 guinea pigs were used at one level of each compound only. An exception was the nitrate; here the two species were used at both levels. The latter were added 5 months after the start of the prolonged exposures.

(c) Animal Diet. The animal diet for a given species was uniform throughout an experiment and, with certain minor exceptions, was maintained without change throughout the entire program. Dogs were fed Purina Dog Chow Kibble Meal mixed with water to which was added 15 per cent Evr Redy Dried Meat with bone to supply additional minerals, notably iron. Cats were fed a mixture of chopped beef and milk; rabbits, oats and Purina Rabbit Chow Checkers; guinea pigs, oats and Rabbit Chow Checkers plus cabbage thrice weekly; rats, Purina Fox Chow; mice, Purina Fox Chow Checkers; and hamsters, Purina Rabbit Pellets and cabbage thrice weekly. Dogs and cats were fed once daily; all other animals had food available at all times except when in the chamber. Water was available at all times except for the aforementioned restriction.

The rat diet showed an increase in fluoride content from approximately 15 ppm in January 1944 to 25 ppm in 1946. Occasional lots that were discarded contained nearly 80 ppm. This gradual increase in fluoride content of the diet necessitated careful controls in experiments in which fluoride storage was measured such as in exposures to uranium tetra- and hexafluorides. Foods with quantities of fluoride greater than 15 ppm considerably increase the normal bone fluoride storage leading to clinical dental fluorosis. The fluoride content of the Rochester water supply is less than 0.1 ppm.

The rate of weight gain of the rats on the wartime diet was not so great as in former periods, indicating an inferior quality of the diet.

(d) Animal Controls. Controls were employed for both acute and chronic studies. According to type of assignment, the controls were designated as "specific" and "nonspecific" controls. Specific controls were animals used for one particular study only. These varied in number according to the needs of the experiment. Nonspecific controls were animals typical in age and weight of animals used in experimental study and served as controls in several experiments progressing more or less synchronically. The purpose of the nonspecific controls was to conserve animal numbers. A group of controls were kept for 90 days and served for a number of studies, each 30 days in duration and not all started simultaneously. Both types of controls were used in the short-term studies; for prolonged studies, however, only specific controls were used.

(1) Short-term Control Studies. Two nonspecific control groups were set up, each 90 days in duration, so that this period overlapped that of a number of concurrently progressing 30-day studies. In one of these studies, 25 animals, comprising dogs, rats, and rabbits, were used, and in the other, 75 animals, comprising dogs, rats, rabbits, guinea pigs, and mice, were used. Animals in these first two control studies were maintained at all times in their cages and had access to food and water. In two subsequent 30-day control studies, animals were exposed in a control-chamber unit under conditions simulating those of the exposure units but without any uranium dust in the atmosphere; 105 animals were used in the first study, 95 in the second. The first study comprised 5 dogs, 25 rabbits, 20 guinea pigs, 25 mice, and 30 rats; the second study used the same numbers of the same species, save 10 fewer rats. A certain proportion of the rabbits and guinea pigs were head exposed in such a way as to serve as controls for two of the units concurrently employing this type of exposure.

(2) Prolonged Control Studies. Serving for the entire 12 one-year exposure studies was a large control-chamber unit. The procedure to which the animals were subjected in this unit was the same as those used for the experimental animals, including extent of handling, number and volume of blood samples, number of tests of toxicologic criteria, and duration of exposure, the only difference being the absence of uranium dust in the atmosphere to which the animals were exposed. Twelve dogs and 175 rats were used. After the exposure of the dogs and rats had progressed through the fifth month, 30 rabbits and 30 guinea pigs were added; 15 each of these two species had only the heads exposed.

(e) Veterinary Service and Animal Maintenance. The procurement of necessarily large numbers of animals for the exposure studies was under the direction of a veterinarian, who passed on the acceptability of animals. On receipt the animals were placed in an observation bay where they were maintained for at least three weeks before use. During this period, dogs and cats were inoculated with antidistemper serum, feces were examined for intestinal parasites, the animals were dewormed if necessary, and the weight and eating habits of the animals were determined. Rabbits were examined for coccidia. When after from 3 to 4 weeks the veterinarian was satisfied as to the suitability of the animals, they were then given to a group in charge of assigning the animals to the various units. During the course of the experimental studies, however, the veterinarian maintained responsibility for the health of the animals. Animals suspected of being in poor condition from causes unrelated to the exposure were taken for diagnosis and treatment. Care was taken that the type of treatment instituted in no way jeopardized the interpretation of the effects related to the exposure to the uranium dusts.

(1) Housing Quarters. Animals were housed for the most part in rooms as far away as possible from the vicinity of the exposure chambers and the dangers of toxic vapors and dusts from other parts of the section. Dogs were kept in specially built rooms. Some of the rats and guinea pigs were maintained in either the same room near the exposure chamber or in interconnecting rooms. Samples of air taken periodically to determine the concentration of uranium and fluoride in the atmosphere, however, showed uniformly negligible amounts and were similar to those found in the control unit. For uranium an average of 0.0006 mg/cu m of air was found (determined by fluorophotometric analysis); for fluoride, values were below the sensitivity of the method, 0.1 ppm.

Dogs were maintained in two-tiered cages constructed of cement with wire floors and doors and with adequate drainage to allow for daily hosing down of the room and cages. The dogs were removed from the room during this operation. A certain number of the dogs were maintained in rooms without such arrangements for drainage and were housed in all-metal cages with wooden shavings for bedding. The dog cages were cleaned daily. All other animals were quartered in all-metal cages of appropriate size arranged in tiers and on casters. Rabbits, except when in the metabolism cages, were maintained on wooden shavings to avoid development of raw hocks; the guinea pigs and mice, and most of the rats, were similarly maintained. Rats, guinea pigs, and mice, the only animals not separately caged, were housed with sufficient space to prevent overcrowding, with provision for adequate ventilation and room for feeding. These animal cages were cleaned three times weekly, but feeding and watering were attended to daily, including Sundays. The temperature and humidity of the animal rooms that were maintained were essentially those of the exposure units. Certain of the rooms were air-conditioned by the same system that conditioned the exposure chambers. In certain instances the humidity in the dog rooms, after steaming out, was higher for short periods than that for the other animals. Because the cages were cleaned thrice weekly with scalding water, sterilization of the cages was not performed more often than once monthly. Floors were washed daily, and corners dusted periodically with DDT powder to prevent accumulation of orthoptera. Such scrupulous cleanliness prevented spread of infection,* minimized body parasites, and maintained animal rooms free from the customary animal odors.

(2) Exposure Cages. All-metal compartmentalized portable cages, with wire screen of ¼-in. to 1-in. mesh (depending on the animal type), were used for the exposure of animals in the chambers. As far as possible the cages were of small-unit size and were on casters to facilitate transportation of the animals from living quarters to exposure chamber. They were equipped with a single tray for the protection of the floor. In certain units, large metal composite cages for dogs, rats, rabbits, and guinea pigs were used.

The exposure chambers and cages were cleaned daily, either by vacuum or by flushing with water. In certain cases, notably cages exposed to uranium tetrachloride and to the uranyl nitrate, special measures for removing the dust from the wire mesh were resorted to. The chloride required scrubbing off with warm water; the nitrate required a bicarbonate bath and subsequent rinsing with water for removal of the bicarbonate.

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* Only one epizootic occurred during the 3 years of operation, a salmonellosis in rats at the start of the prolonged studies. A coccidial infection in rabbits was brought in from a local farm; an epizootic among guinea pigs presumably of viral nature arose in animals from a supply house in Pennsylvania.

(f) Preexposure Examination of Animals. For special studies in which uniform control data were demanded, animals, especially rabbits and dogs, were examined for fitness by special tests of renal sufficiency, such as urinary protein and catalase and blood nonprotein nitrogen. From results of such tests, animals with conditions suggestive of nephritis were eliminated from use. In general, animals of all species were observed likewise for a period of from 3 to 4 weeks before placing them in an experiment, during which time they were marked and weighed and the necessary control data were obtained. After this period, if satisfactory weight gains were observed, the animals were ready for the conditioning period.

(g) Conditioning Period. All animals were given a period of acclimatization to conditions in the exposure unit immediately prior to exposure to the uranium dust. The conditions of restricted action and nonavailability of food and water in the exposure unit for periods of 6 hr daily imposed certain additional stresses on the animal that required some bodily adjustment. The restricted action followed as a consequence of the exposure of each animal in small individual cage compartments. This procedure was employed in order to ensure maximal exposure of each animal and thus avoid bunching and consequent filtering of the dust before breathing by one animal nuzzling into the fur of another. Food and water were omitted, obviously to minimize the ingestion of uranium dust. That the conditioning period in the exposure unit was essential, in addition to the previous 1-month observational period, was shown by weight losses notably in the larger species during the first week of conditioning. Conditioning exposure served also to bring to light latent infection that would otherwise go undetected until the dust exposure had been in progress. Similarly, the weight losses occasioned by the effects of exposure conditions would otherwise be confused with those resulting from the effects of uranium exposure.

The conditions during this period were the same as those ultimately employed in the dust study. The period of exposure was for 6 hr daily, except Sundays, and for 2 weeks if the animals were assigned for a 30-day study; 4 weeks (28 calendar days), if for one 1-year study. These periods usually sufficed to ensure the animal's adjustment, regain in weight, discovery of latent infection, and general fitness for study throughout the experimental term.

(h) Exposure Period. Thirty exposure days were employed uniformly for the short-term studies, although at the start a few levels were tested for 1 calendar month, or a somewhat shorter period. Animals were exposed on a schedule of 6 hr a day, 6 days a week in so far as possible so that the effects of the dust were tested for a total time interval of 180 hr over a period of approximately 5 calendar weeks. At the time when animals were exposed in two shifts per day, the same schedule was maintained, the low dust level being used on the first shift, the higher level on the second shift. In order to equalize the exposures of both shifts, each level was exposed on alternate Saturdays allowing an average exposure time of each level of 33 hr per week.

The prolonged studies were designed for 1 calendar year of exposure. Because all these studies were performed on a daily two-shift basis, as well as the later short-term runs, the same schedule of exposure hours was followed in these studies permitting 33 hr weekly exposure for the animals at each level.

(i) Types of Biological Observations. In addition to the periodic determinations of weight of the animals, of chemical analysis of blood and urine, hematologic examinations, terminal pathology examinations, and uranium analyses of tissues that were routinely employed in both acute and chronic studies, certain special measures were used in the chronic studies, but in some cases they were employed for both types of study. The studies to which each measure was applied follow.

(1) Serial Histologic Examination. Perhaps the most informative of the special procedures was that of serial killing and histologic examination of the animals while the experiment was in progress. Such a procedure allowed the course of toxicity to be traced at critical intervals and was accordingly adopted uniformly for both chronic and acute studies. The animals and the time of scheduled sacrifice differed according to the study. In the acute study, only rats and certain rabbits (and mice in the uranium tetrachloride studies) underwent this procedure. Depending upon the type of information desired, rats were examined at a rate of three per day per week, or one per day each 30 days of the study, with the addition of certain other animals at critical periods. In the chronic study, dogs, rats, and certain rabbits were sacrificed. One dog at each level of exposure was sacrificed at 10 days and at 4, 6, and 8 months. Three rats were sacrificed weekly for the first 4 weeks, and semimonthly or monthly thereafter throughout the course of the experiment, with more frequent sacrifices during the first week. Analyses for uranium content were uniformly made on tissues from all sacrificed animals in the chronic studies, although this was not done for animals in the acute studies.

(2) Serial Renal Biopsy of the Dog. While the procedure of serial sacrifice allowed only one observation per animal on the type of toxicity developed from exposure to the dust, the procedure of serial biopsy of the kidney, and in one instance of the liver, allowed the progress of the toxicity to be followed in the same animal. For this procedure only dogs were used and, although it was applied uniformly to the chronic studies, only certain of the later short-term studies made use of this technique. In the latter studies one dog was biopsied before the start of exposure and once again during the 30 exposure days. In the chronic studies two dogs were used at each level, and, in addition to the original biopsy that was performed at least 1 month prior to the experiment, biopsies occurred after 10 days, and after 4 and 8 months of exposure. Analyses for uranium content were likewise made on these tissues at the time of biopsy.

(3) Microscopic Examination of Urine. In order to secure information as to the extent of recovery from the biopsy, a microscopic examination of the urine for the number of red and white blood cells and the number and type of casts was made prior to each biopsy. In addition, determinations were made in five catheterized dogs in which renal clearances were studied on the morning of the day of the experiment. Urine of five dogs used in the control unit was examined microscopically once a month. As a guide in the evaluation of the results, values exceeding 10,000 WBC, 100,000 RBC, and 2,000 casts were considered abnormal.

In the hands of an experienced operator this procedure was extremely successful. Of 96 biopsies only five fatalities have occurred that were directly attributable to the procedure. The details of the technique follow.

The purpose of the operation was to examine the kidney grossly in vivo and to obtain a specimen of the kidney for microscopic study. Preoperative medication consisted of morphine sulfate in therapeutic amounts depending upon the weight of the animal (about 1/150 grain) and ¼ grain of atropine sulfate. The anesthetic was pentothal sodium administered in amounts dependent upon the size of the animal and operative procedure. Skin was prepared for operation with soap, water, alcohol, and metaphen.

Procedure. (Paul E. Rekers.) The animal is postured with the left flank up. The left flank is hyperextended by means of a built-in kidney lift on the operating table, projecting into the right flank of the animal. Following skin preparation and draping of the animal, an incision 7 to 8 cm in length is made in the left flank parallel to and 2 to 3 cm below the twelfth rib. The skin edges are walled off. The incision is then deepened by dissection through the individual muscle layers following the course of the muscle fibers. In the case of the internal oblique muscle, the fascia is split by a sharp dissection. When the peritoneum is opened, the entire wound is walled off by means of pads moistened with physiological saline. The kidney is then examined in situ undisturbed.

Following the examination, the kidney is delivered into the wound and surrounded by means of pads well-moistened with physiological saline. By means of a sharp razor a wedge of kidney tissue, measuring approximately 2 to 2½ cm on the cortical surface, ½ to 2 cm in length, and 3 to 5 mm in thickness and weighing approximately 1 to 1½ g, is removed from the upper pole of the kidney. This specimen represents a radial section extending from the kidney cortex down to and through the renal surface. A removed kidney specimen receives a minimal amount of handling. The fresh-cut edges of the kidney are approximated by means of multiple, alternating mattress and interrupted sutures of renal size 4-0 with an atraumatic 1-in. needle. Hemostasis is adequately affected by this procedure. Digital occlusion of the renal artery and vein is employed at the time of removing the specimen and the suturing of the surgical defect.

In some instances a few buried mattress sutures deep in the kidney substance are necessary. The kidney is returned to its normal bed covered with the usual perirenal fat. The operative flank wound is closed in layers, lock-stitched in the peritoneum with interrupted sutures for muscles, superficial fascia, subcutaneous region, and skin. Fine silk is used. The peritoneal layer overlying two muscle layers is sutured with surgeon's silk twist, size 3-0, on a French spring-eye half-circle size 2 needle (see Fig. 10.30). The subcuticular layer is sutured with plain catgut, size 3-0, on a straight triangular-point (1¾ in. in length) surgical needle using continuous suture. Skin sutures of catgut are preferentially used. A small dressing is applied to the skin and held in place by means of liquid adhesive tape. Approximately 100 ml of physiological saline solution is administered intravenously during the operative procedure. At the close of the procedure the animal is usually semiconscious and moves about. The appearance of a normal kidney at different stages of the operation is illustrated in Fig. 10.30.

Following operation, the animal was carefully placed on its side in a separate cage and kept warm with covers. The animal was turned each half hour to avoid pulmonary congestion; and if the animal had not regained consciousness within 1 hr following the operation it was given an injection of 1.5 ml of coramine. The animal was observed closely for the next 12 hr, and reports on its condition were made periodically to the surgeon until the final sacrifice of the animal.

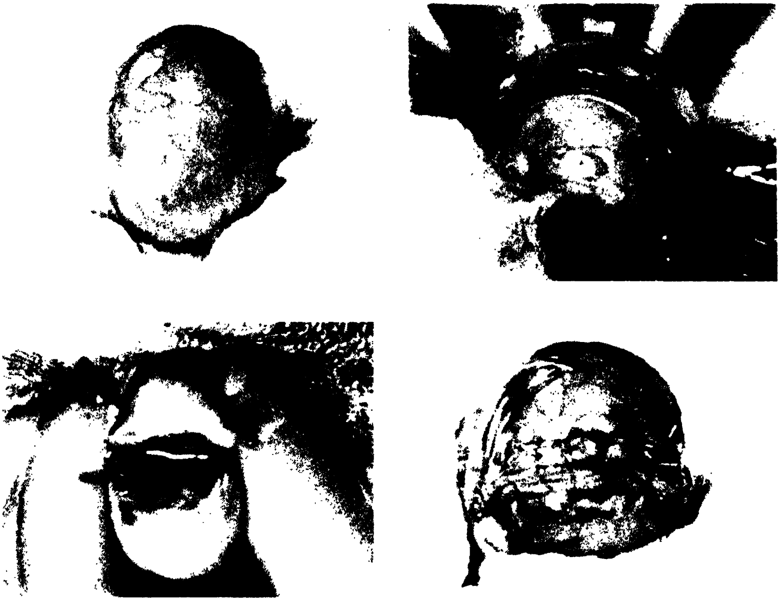

Fig. 10.30 — Photographs illustrating major steps in the renal biopsy of the dog. Upper left, kidney delivered from renal bed; upper right, kidney with wedge of cortex and pelvis removed; lower left, another view of kidney with wedge removed; lower right, renal wound closed, kidney ready for replacement in renal bed.

(4) Renal-clearance Study in the Dog. Because the kidney was considered to be the chief organ of attack by the uranium materials, the effects of these agents on the physiological function of this organ were studied. The ability of the kidney to clear the blood of certain naturally occurring and injected materials has been well studied in some species. For the inhalation studies the dog was used. Diodrast, inulin, and chloride were the materials for which clearance was measured (see Chap. 5 for the significance of renal clearance).

This procedure was confined to female animals exposed to the 0.3 and 1.9 mg/cu m levels of uranyl nitrate. Three animals were used at the lower dust level, and two at the higher one. Several control clearance values were obtained prior to the exposure of the animals in the chamber. Following exposure, clearance of the three constituents were determined after the first week of exposure and biweekly thereafter. The procedure of renal clearance as applied to the dog is described in detail below.

The procedure consists of (1) collecting the urine specimen prior to the clearance for chemical and microscopic examination to determine the suitability of the animal for the experiment, (2) collection of a blood sample for preclearance blood analysis, (3) preparation, which consists of a priming period of 45 min, a 60-min infusion period, and four 15-min periods for the determination of the clearances.

Preparation of the dog for the experiment consisted of catheterizing for a preinfusion urine specimen. For this a wing-tip soft rubber catheter and a metal probe were used, the catheter being left in position. One milliliter of this specimen was used for the determination of catalase activity, and the remainder for the determination of protein, amino acid nitrogen, creatinine, and a microscopic examination. A preinfusion oxalated blood sample was obtained from the jugular vein for analysis of nonprotein nitrogen. At this time a complete blood count was made. The dog was then placed on its side and immobilized with four leg ties and a shoulder-band restrainer. The animal was covered to compensate for decreased body temperature.

The infusion apparatus (see Chap. 5 for description) is prepared by flushing the pump with alcohol-salicylic acid solution (0.1 per cent salicylic acid in 80 per cent alcohol) and rinsing with sterile distilled water followed by a rinse with sterile priming fluid. The machine is set to deliver 3 ml/min. Infusion of the animal is performed by inserting a No. 20 1-in. needle with syringe into a hind-leg vein injecting slowly 2 to 3 ml of priming fluid. The syringe is replaced by the delivery tube from the pump and infusion is continued for exactly 45 min. At this time the priming fluid is replaced with sterile infusion fluid, which is injected for 60 min. At the end of the 60-min period, urine sample 1 is collected in a tared flask for a period of 15 min. After 7 ½ min, a 7-ml sample of venous blood in oxalate is taken; the same procedure is repeated for three additional 15-min periods for both urine and blood samples that are analyzed for inulin, diodrast, and chloride.

Solutions

1. Primer:

Glucose	10.0 g
Inulin	7.0 g
35 % diodrast solution (70 % diluted with distilled H_2O)	14.0 ml
NaCl	6.8 ml
Na_2SO_4	20.0 ml
H_2O q.s.	1,000.0 ml

2. Infusion:

Glucose	10.0 g
Inulin	3.5 g
35 % diodrast solution (70 % diluted with distilled H_2O)	7.0 ml
NaCl	6.8 ml
Na_2SO_4	20.0 ml
H_2O q.s.	1,000.0 ml

(5) Liver-function Blood-coagulation Test. In an effort to determine to what degree the liver may be affected either directly or indirectly by the inhalation of uranium dusts, three tests of hepatic function were applied to certain dogs and rats and to occasional rabbits exposed to the nitrate, hexafluoride, and tetrachloride products. The tests used were the determination of blood fibrinogen levels, prothrombin clotting time, and percentage of bromsulfalein dye retention (see Chap. 5 for discussion of these tests). All three tests were used uniformly in dogs on the afore-mentioned indicated dusts during the period of the chronic studies; the prothrombin and fibrinogen only were determined in rats and rabbits. On chronic studies, control values were established prior to the dust exposure, as well as in control animals, and subsequently tests were made simultaneously during exposure at week 1 during the first month of conditioning, and during weeks 1, 2, and 4 of month 2, and at monthly intervals thereafter. These tests were limited to but one level each of the tetrachloride and hexafluoride exposure of the short-term studies.

Although the method involves heart punctures of the rat, in the hands of an experienced technician fatalities are reduced to 2 or 3 per cent based on the total number of heart punctures.

(6) Tissue Analysis. Uranium Content. The practice of determining the distribution and content of uranium in the tissues of animals exposed to the dust has been uniformly applied to animals of both the short- and long-term studies. In the short-term studies the spectrographic method* of analysis was employed throughout. Frequently the tissues of six species of animals were analyzed by this method following the conclusion of each experiment and, in some instances, during exposure and also at various intervals after the termination of the exposure. In the chronic studies, the tissue content of uranium was determined by the fluorophotometric method (described in Chap. 2), a more sensitive method for the determination of uranium. Similarly to the short-term studies, the chronically exposed

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* Method described in Chap. 2.

animals were analyzed terminally, but in addition the uranium content of tissues was established both in dogs and rats that were killed serially throughout the course of the experiment, thus affording a correlation of the uranium content with the pathology of a given tissue. Two rats were killed on each of the first 10 days of exposure, at 2-week intervals for the next 2 months, and monthly thereafter throughout the course of the experiment. The tissues were analyzed for uranium. At the end of the exposure, 25 rats were killed in a group for similar examinations. The lung, kidney, liver, and femoral bone of these animals were analyzed. At certain widely spaced intervals 20 tissues of two rats were analyzed separately. Approximately 20 tissues of each dog at each level were analyzed at the scheduled period of histologic examination and the lung, liver, kidney, and bone of the entire number of 20 dogs were analyzed terminally. The kidneys of a certain number of rabbits and guinea pigs were analyzed terminally also.

Fluoride Content. Certain hard tissues of the rats exposed in the chronic studies at higher levels of the tetrafluoride and hexafluoride dusts were analyzed for the fluoride content at periodically scheduled intervals throughout the course of the exposure.

(7) Dental Examination. Dental examination of the condition of rats' teeth following exposure to the uranium compounds containing fluoride was made in an effort to correlate the results with the fluoride and uranium content of the teeth and with other osseous structures. Such examinations were performed on animals in both the short- and long-term studies.

(8) Dying Animals. An autopsy was performed on all dying animals that were not scheduled for histologic examination in order to establish as far as possible the cause of unexpected deaths and to check otherwise undetected infection.

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